510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY AND INSTRUMENT COMBINATION TEMPLATE

A. 510(k) Number:

K132270 Roche **cobas**® CT/NG v2.0 Test

B. Purpose for Submission:

To determine substantial equivalence for the **cobas**® CT/NG v2.0 Test for detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* DNA from self-collected vaginal swabs, clinician-collected vaginal swabs, endocervical swabs, male and female urine, and cervical specimens collected in PreservCyt solution. Differences between the previously cleared **cobas**® CT/NG Test (K110923) and the **cobas** CT/NG v2.0 Test include additional specimen types and modification of the sample preparation workflow which requires a **cobas** 4800 system software update. There are no changes to the assay reagents.

C. Measurand:

Chlamydia trachomatis DNA

Neisseria gonorrhoeae DNA

D. Type of Test:

Qualitative *in vitro* diagnostic assay that utilizes amplification of target DNA by real-time Polymerase Chain Reaction.

E. Applicant:

Roche Molecular Systems, Inc.

F. Proprietary and Established Names:

Roche **cobas®** CT/NG Test and **cobas®** 4800 System

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.3120 Chlamydia serological reagents
   21 CFR 866.3390 Neisseria spp. direct serological test reagents
   21 CFR 862.2570 Instrumentation for Clinical Multiplex Test Systems

2. Classification:

   Class II

3. Product code:

   MKZ: DNA Probe, Nucleic Acid Amplification, Chlamydia
   LSL: DNA-Reagents, Neisseria
   OOI: Real Time Nucleic Acid Amplification System

   Panel:

   Microbiology 083

H. Intended Use:

1. Intended use(s):

   Assay:

   The **cobas**® CT/NG v2.0 Test is an automated, in vitro nucleic acid amplification test for the qualitative detection of *Chlamydia trachomatis* (CT) and/or *Neisseria gonorrhoeae* (NG) DNA in urogenital specimens. The Test utilizes the Polymerase Chain Reaction (PCR) for the detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* DNA in male and female urine, self-collected vaginal swab specimens (collected in a clinical setting), clinician-collected vaginal swab specimens, and endocervical swab specimens, all collected in **cobas**® PCR Media (Roche Molecular Systems, Inc.), and cervical specimens collected in PreservCyt® solution. This test is intended as an aid in the diagnosis of chlamydial and gonococcal disease in both symptomatic and asymptomatic individuals.

   Ancillary Collection Kits:

   The **cobas**® PCR Female Swab Sample Kit is used to collect and transport endocervical and vaginal swab specimens. The **cobas**® PCR Media serves as a nucleic acid stabilizing transport and storage medium for gynecological specimens. Use this collection kit only with either the **cobas**® CT/NG Test or the **cobas**® CT/NG v2.0 Test.

   The **cobas**® PCR Urine Sample Kit is used to collect and transport urine specimens. The **cobas**® PCR Media serves as a nucleic acid stabilizing transport and storage medium for urine specimens. Use this collection kit only with either the **cobas**® CT/NG Test or the **cobas**® CT/NG v2.0 Test.

2. Indication(s) for use:

   Same as Intended Use

3. Special conditions for use statement(s):

   For prescription use only

4. Special instrument requirements:

   cobas® 4800 System

I. Device Description

The **cobas®** CT/NG 4800 System is a multi-instrument platform that performs qualitative *in vitro* nucleic acid amplification tests from human specimens. The system integrates automated total nucleic acid isolation, PCR setup, and real-time PCR. The **cobas** CT/NG 4800 System consists of the **cobas x** 480 Instrument for specimen preparation, and the **cobas z** 480 Analyzer for amplification and detection. The **cobas** 4800 system software integrates sample preparation with nucleic acid amplification and detection to generate test results.

The **cobas** 4800 System maintains positive identification for each specimen during processing and analysis by barcodes. Controls are also identified and tracked via barcodes.

The **cobas x** 480 Instrument provides automated sample preparation in a PCR microwell plate in batch sizes of 24 or 96 samples/controls. Samples are pipetted from the specimen tubes and undergo preparation to extract nucleic acids. Extracted sample eluate is combined with working master mix reagent in a barcoded PCR microwell plate, after which the plate is sealed and transferred to the **cobas z** 480 analyzer for automated PCR amplification and detection.

The following reagent kits are used when performing the **cobas** CT/NG v2.0 Test:
cobas® 4800 System Sample Preparation Kit
cobas® 4800 CT/NG v2.0 Amplification/Detection Kit
cobas® 4800 CT/NG Controls Kit
cobas® 4800 System Wash Buffer Kit
cobas® 4800 System Control Diluent Kit
cobas® 4800 System Liquid Cytology Preparation Kit

Sample Collection Kits to be used with the **cobas**® CT/NG Test are:
cobas® PCR Female Swab Sample Kit (for endocervical and vaginal swabs)
cobas® PCR Urine Sample Kit
PreservCyt®

J. Substantial Equivalence Information:

1. Predicate device name(s):

   cobas CT/NG Test, Roche Molecular Systems

2. Predicate 510(k) number(s):

K110923

3. Comparison with predicate:

Similarities		
Item	**cobas CT/NG v2.0 Test**	**Predicate Device: cobas CT/NG Test (K110923)**
Intended Use	Qualitative in vitro diagnostic test for the direct qualitative detection of *Chlamydia trachomatis* and/or *Neisseria gonorrhoeae* in patient specimens	same
Symptomatic Status	Asymptomatic and symptomatic	same
Sample Preparation Procedure	Semi-automated	same
CT Analyte Targets	CT cryptic plasmid DNA CT *omp*A gene	same
NG Analyte Targets	NG genomic DNA	same
Amplification Technology	Real-time PCR	same
Detection Chemistry	Paired reporter and quencher fluorescence labeled probes (TaqMan Technology) using fluorescence resonance energy transfer (FRET)	same
Result Analysis	Based on PCR cycle threshold (Ct) analysis	same

Differences		
Item	**cobas CT/NG v2.0 Test**	**Predicate Device: cobas CT/NG Test (K110923)**
Specimen Types	Male urine Female urine Endocervical swabs Clinician-collected vaginal swabs Patient-collected vaginal swabs Cervical specimens in PreservCyt® Solution	Male urine Patient-collected vaginal swabs
Sample Collection Devices	**cobas** PCR Urine Sample kit **cobas** PCR Female Sample Kit PreservCyt® Solution	**cobas** PCR Urine Sample kit **cobas** PCR Female Sample Kit

K. Standard/Guidance Document Referenced (if applicable):

N/A

L. Test Principle:

The **cobas** CT/NG v2.0 Test for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* is based on two processes: (1) automated sample preparation to obtain CT and NG DNA; (2) simultaneous PCR amplification of target DNA sequences using both CT and NG specific primer pairs and real-time detection of cleaved fluorescent-labeled CT and NG specific oligonucleotide detection probes. An Internal Control, containing CT and NG DNA, is added to all samples prior to automated sample preparation and is amplified and detected simultaneously with each sample to monitor the entire process.

Sample preparation for the **cobas** CT/NG v2.0Test is automated with the use of the **cobas x** 480 instrument. Specimens are lysed in the collection device by the chaotropic agent in the **cobas** PCR Media. Released nucleic acids, along with added CT/NG Internal Control DNA, are purified through binding to magnetic glass particles, washed, and separated from these particles making them ready for PCR amplification and detection.

The Master Mix reagent contains primer pairs and probes specific for the CT cryptic plasmid DNA, the CT genomic *omp*A gene DNA, NG genomic DNA sequences A and B within the DR-9 region, and CT and NG Internal Control DNA.

Target Selection

The **cobas** CT/NG v2.0 Test detects a DNA sequence in the cryptic plasmid common to all serovars of *C. trachomatis* as well as a separate DNA sequence within the *C. trachomatis* chromosome. For *N. gonorrhoeae*, the test detects two targeted sequences in a highly conserved direct repeat region.

Target Amplification

Processed samples are added to the amplification mixture in a microwell plate in which PCR amplification occurs. The reaction mixture is heated to separate the isolated double-stranded DNA and expose the primer target sequences. As the mixture cools, the primers anneal to the target DNA. Z05 DNA polymerase, in the presence of Mn^{2+} and excess dNTPs, extends the annealed primers along the target templates to produce double-stranded DNA. This completes the first cycle of PCR, yielding a double-stranded DNA copy of the target regions of the CT and/or NG DNA and the CT/NG Internal Control DNA. Repetition of this process results in the amplification of DNA between the primer target sequences, producing a double-stranded DNA molecule termed an amplicon. The **cobas z** 480 analyzer automatically repeats this process for a designated number of cycles, with each cycle intended to double the amount of amplicon DNA.

Internal Control Amplification

The CT/NG Internal Control is a combination of two non-infectious recombinant plasmid DNAs, each with primer binding regions identical to those of either the *C. trachomatis* or the *N. gonorrhoeae* genomic target sequences. Both recombinant plasmid DNAs have an identical randomized internal target sequence, and a unique probe binding region that differentiates the CT/NG Internal Control from target amplicon. These features were selected to ensure independent detection of both the CT/NG Internal Control and the *C. trachomatis* and *N. gonorrhoeae* target DNAs. The CT/NG Internal Control Reagent is included in the **cobas** CT/NG v2.0 Test and is introduced into each sample on the **cobas x** 480 instrument during sample processing.

Selective Amplification

Selective amplification of target nucleic acid from the specimen is achieved in the cobas CT/NG v2.0 Test by the use of AmpErase (uracil-N-glycosylase) enzyme and deoxyuridine triphosphate (dUTP). The AmpErase enzyme recognizes and catalyzes the destruction of DNA strands containing deoxyuridine, but not DNA containing deoxythymidine. Deoxyuridine is not present in naturally occurring DNA, but is always present in amplicon due to the use of deoxyuridine triphosphate in place of thymidine triphosphate as one of the dNTPs in the Master Mix reagent; therefore, only amplicon contain deoxyuridine. Deoxyuridine renders contaminating amplicon susceptible to destruction by AmpErase enzyme prior to amplification of the target DNA. AmpErase enzyme, which is included in the Master Mix reagent, catalyzes the cleavage of deoxyuridine-containing DNA at the deoxyuridine residues by opening the deoxyribose chain at the C1-position. When heated in the first thermal cycling step at the alkaline pH of Master Mix, the amplicon DNA chain

breaks at the position of the deoxyuridine, thereby rendering the DNA non-amplifiable. AmpErase enzyme is inactive at temperatures above 55ºC, i.e., throughout the thermal cycling steps, and therefore does not destroy target amplicon. The **cobas** CT/NG v2.0 Test has been demonstrated to inactivate at least 10^3 copies of deoxyuridine-containing CT/NG amplicon per PCR.

Detection of PCR Products

The **cobas** CT/NG v2.0 Test utilizes real-time PCR technology. The use of fluorescent probes enables real-time detection of PCR product accumulation by monitoring the emission intensity of fluorescent dyes released during the amplification process. The probes include CT cryptic plasmid, CT *ompA*, NG DR-9A, NG DR-9B and CT/NG Internal Control-specific oligonucleotides, all labeled with a reporter dye and a quencher. When the fluorescent dye-labeled probes are intact, the reporter fluorescence is suppressed by the proximity of the quencher due to Förster-type energy transfer effects. During PCR, the probes hybridize to their respective target sequence and are cleaved by the 5' to 3' nuclease activity of the thermostable Z05 DNA polymerase. Once the reporter and quencher are separated, quenching no longer occurs, and the fluorescent emission of the reporter dyes increases. The amplification of CT targets, NG targets and the CT/NG Internal Control are measured independently and at different wavelengths. This process is repeated for a designated number of cycles, each cycle increasing the emission intensity of the individual reporter dyes.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision:

Precision of the **cobas** CT/NG v2.0 Test was evaluated in-house using a panel composed of CT and NG cultures diluted into the following three natural specimen matrices that were predetermined to be negative for both CT and NG prior to spiking:

- Pooled negative vaginal swab specimen matrix in **cobas** PCR Media
- Pooled negative urine in **cobas** PCR Media
- Pooled negative PreservCyt specimens.

The precision panel for each of the three matrices contained one negative panel member and three positive panel members containing combinations of CT and NG at 1X and 3X LOD. Testing was performed with three lots of **cobas** CT/NG v.2.0 Test reagents in 24 runs on three instruments over 12 days. A total of 48 replicates were tested for each panel member. Study results demonstrated the expected hit rates for all positive panel members and 100% negative results for all replicates of the negative panel member. Analysis of variance of Cycle Threshold (Ct) values from valid tests performed on positive panel members demonstrated overall Coefficient of Variation (CV) % ranges from 1.0% to 3.0% for CT and from 1.0% to 2.0% for NG. Detailed results for this study are presented in the tables below.

In-House Precision Study Hit Rate Analysis

		CT					NG				
Panel Matrix	**Panel Member**	**Target Level**	**Positive**	**Valid**	**% Hit Rate**	**95% CI**	**Target Level**	**Positive**	**Valid**	**% Hit Rate**	**95% CI**
Vaginal	1	0	0	48	0	0 - 7.4%	0	0	48	0	0 - 7.4%
	2	1 x LOD	48	48	100	92.6 - 100%	1 x LOD	48	48	100	92.6 - 100%
	3	3 x LOD	48	48	100	92.6 - 100%	1 x LOD	48	48	100	92.6 - 100%
	4	1 x LOD	47	48	98	88.9 - 99.9%	3 x LOD	48	48	100	92.6 - 100%
		CT					**NG**				
Panel Matrix	**Panel Member**	**Target Level**	**Positive**	**Valid**	**% Hit Rate**	**95% CI**	**Target Level**	**Positive**	**Valid**	**% Hit Rate**	**95% CI**
Urine	1	0	0	48	0	0 - 7.4%	0	0	48	0	0 - 7.4%
	2	1 x LOD	48	48	100	92.6 - 100%	1 x LOD	48	48	100	92.6 - 100%
	3	3 x LOD	48	48	100	92.6 - 100%	1 x LOD	48	48	100	92.6 - 100%
	4	1 x LOD	48	48	100	92.6 - 100%	3 x LOD	48	48	100	92.6 - 100%
		CT					**NG**				
Panel Matrix	**Panel Member**	**Target Level**	**Positive**	**Valid**	**% Hit Rate**	**95% CI**	**Target Level**	**Positive**	**Valid**	**% Hit Rate**	**95% CI**
PreservCyt	1	0	0	47	0	0 - 7.5%	0	0	47	0	0 - 7.5%
	2	1 x LOD	48	48	100	92.6 - 100%	1 x LOD	47	48	98	88.9 - 99.9%
	3	3 x LOD	47	47	100	92.5 - 100%	1 x LOD	47	47	100	92.5 - 100%
	4	1 x LOD	48	48	100	92.6 - 100%	3 x LOD	48	48	100	92.6 - 100%

CT Precision: Overall Mean, Standard Deviations and Coefficients of Variation (%) for Cycle Threshold Values

Panel Member	n^1/N	Mean Ct	SD Components / CV%						
			System	Lot	Operator	Day	Run	Random	Total
Vaginal Swab Specimen in cobas PCR Media									
2	48/48	36.5	0.000	0.247	0.000	0.095	0.000	0.398	0.478
			0%	1%	0%	0%	0%	1%	1%
3	48/48	35.8	0.192	0.000	0.000	0.000	0.250	0.310	0.442
			1%	0%	0%	0%	1%	1%	1%
4	47/48	36.7	0.000	0.067	0.000	0.000	0.000	0.674	0.678
			0%	0%	0%	0%	0%	2%	2%
Urine in cobas PCR Media									
2	48/48	35.5	0.147	0.058	0.000	0.000	0.000	0.335	0.370
			0%	0%	0%	0%	0%	1%	1%
3	48/48	34.6	0.100	0.077	0.062	0.000	0.000	0.184	0.232

			0%	0%	0%	0%	0%	1%	1%
4	48/48	35.4	0.145	0.161	0.000	0.000	0.243	0.295	0.439
			0%	0%	0%	0%	1%	1%	1%
Cervical Specimen in PreservCyt Solution									
2	48/48	35.0	0.289	0.365	0.000	0.000	0.539	0.815	1.082
			1%	1%	0%	0%	2%	2%	3%
3	47/47	33.7	0.234	0.000	0.000	0.000	0.385	0.420	0.616
			1%	0%	0%	0%	1%	1%	2%
4	48/48	34.7	0.000	0.000	0.000	0.420	0.287	0.655	0.830
			0%	0%	0%	1%	1%	2%	2%

NG Precision: Overall Mean, Standard Deviations and Coefficients of Variation (%) for Cycle Threshold Values

Panel Member	n^1/N	Mean Ct	SD Components / CV%						
			System	Lot	Operator	Day	Run	Random	Total
Vaginal Swab Specimen in cobas PCR Media									
2	48/48	37.1	0.000	0.211	0.000	0.000	0.000	0.583	0.620
			0%	1%	0%	0%	0%	2%	2%
3	48/48	37.2	0.252	0.185	0.000	0.052	0.000	0.509	0.600
			1%	0%	0%	0%	0%	1%	2%
4	48/48	36.5	0.152	0.000	0.000	0.000	0.000	0.417	0.444
			0%	0%	0%	0%	0%	1%	1%
Urine Plus cobas PCR Media									
2	48/48	36.2	0.000	0.181	0.000	0.000	0.000	0.456	0.491
			0%	0%	0%	0%	0%	1%	1%
3	48/48	36.1	0.000	0.159	0.052	0.000	0.134	0.337	0.400
			0%	0%	0%	0%	0%	1%	1%
4	48/48	35.1	0.143	0.229	0.000	0.000	0.182	0.281	0.430
			0%	1%	0%	0%	0%	1%	1%
Cervical Specimen in PreservCyt Solution									
2	47/48	34.7	0.000	0.311	0.000	0.000	0.000	0.797	0.855
			0%	1%	0%	0%	0%	2%	2%
3	47/47	34.8	0.000	0.000	0.000	0.000	0.587	0.606	0.844
			0%	0%	0%	0%	2%	2%	2%
4	48/48	33.5	0.124	0.000	0.000	0.000	0.540	0.618	0.830
			0%	0%	0%	0%	2%	2%	2%

Additional in-house precision testing was performed with samples containing CT and NG concentrations below the LoD (high negative samples). Panel members were prepared by spiking CT and NG cultures into a pool of vaginal specimen matrix collected in **cobas** PCR Media, a pool of urine matrix stabilized in cobas® PCR Media and a pool of cervical specimen matrix collected in PreservCyt Solution to organism levels designed to give a negative result approximately 20 to 80% of the time. Negative panel members were also prepared for each matrix. For each sample matrix, panels were tested over the

course of 12 days by two operators using three lots of reagents and two **cobas** 4800 Systems. Two replicates of each panel member were tested in each run, generating up to 48 test results for each panel member. Testing of the high-negative panel member yielded the anticipated hit rate for all three matrices.

In-House Precision Study Hit Rate Analysis for High –Negative Levels

Vaginal Swab Specimen Collected in cobas PCR Media										
Panel	**Levels**		**CT**				**NG**			
	CT	**NG**	**Positive**	**Valid**	**Hit Rate (%)**	**95% CI**	**Positive**	**Valid**	**Hit Rate (%)**	**95% CI**
1	Neg	Neg	0	48	0	0 - 7.4	0	48	0	0 - 7.4
2	Neg	HNeg	0	48	0	0 - 7.4	29	48	60	45.3 - 74.2%
3	HNeg	Neg	22	48	46	31.4 - 60.8%	0	48	0	0 - 7.4
Urine Stabilized in cobas PCR Media										
Panel	**Levels**		**CT**				**NG**			
	CT	**NG**	**Positive**	**Valid**	**Hit Rate (%)**	**95% CI**	**Positive**	**Valid**	**Hit Rate (%)**	**95% CI**
1	Neg	Neg	0	48	0	0 - 7.4	0	48	0	0 - 7.4
2	Neg	HNeg	0	48	0	0 - 7.4	32	48	67	51.6 - 79.6%
3	HNeg	Neg	40	48	83	69.8 - 92.5%	0	48	0	0 - 7.4
Cervical Specimen Collected in PreservCyt Solution										
Panel	**Levels**		**CT**				**NG**			
	CT	**NG**	**Positive**	**Valid**	**Hit Rate (%)**	**95% CI**	**Positive**	**Valid**	**Hit Rate (%)**	**95% CI**
1	Neg	Neg	0	48	0	0 - 7.4	0	48	0	0 - 7.4
2	Neg	HNeg	0	47	0	0 - 7.5	26	47	55	40.1 - 69.8%
3	HNeg	Neg	26	47	55	40.1 - 69.8%	0	47	0	0 - 7.5

Neg = negative level
HNeg = high negative level

b. Reproducibility:

A Reproducibility Study was performed across testing sites, operators, runs, and days for the **cobas** CT/NG v2.0 Test using panels of samples separately prepared in the following natural specimen matrices that were predetermined to be negative for both CT and NG prior to spiking:

- Pooled negative vaginal swabs collected in **cobas** PCR Media
- Pooled negative urine matrix stabilized in **cobas** PCR Media

- Pooled negative cervical specimen matrix collected in PreservCyt Solution.

The positive panel members for each matrix contained CT or NG at concentrations of 1 x LOD and 3 x LOD. Also included was one negative sample containing matrix only. Testing was performed at two external sites and one in-house site. A run for **cobas** PCR Media (urine and swab) included three replicates of each of five panel members and one positive and one negative control (32 total tests). A run for the PreservCyt Solution panel included three replicates of each of five panel members and one positive and one negative control (17 total tests). Two operators at each site performed one run per day each, for a total of five days of testing per operator per panel type (10 days of testing total for each panel type). Testing was performed with one reagent lot.

Overall, 127 runs were performed: 62 included a combination of urine and swab samples and 65 included only PreservCyt samples. Seven failed runs (two runs with urine/swab samples and five runs with PreservCyt samples) were attributed to protocol deviations and instrument errors. There was one invalid test result in a PreservCyt sample and one failed test result each in swab, urine and PreservCyt samples. These failed tests were due to instrument errors.

All valid test results were included in the separate analyses of the percent agreement for CT and NG for each panel type. For negative panel members, there were no false positive results for all three matrices thus resulting in negative percent agreement (NPA) of 100% for both CT and NG.

Reproducibility Results for CT:

Testing resulted in 100% positive percent agreement for samples prepared in all three matrices containing CT concentrations at 3X LOD as well as samples prepared in a urine matrix with CT concentrations at 1X LOD. Samples prepared in vaginal swab and PreservCyt matrices containing CT at 1X LOD demonstrated 96.7% and 98.9% positive percent agreement. Analysis of variance components of Ct values for CT-positive panel members demonstrated overall CV's ranging from 1.4% to 2.6% for all three matrices. Detailed results from testing are presented in the following tables:

***C. trachomatis*: Overall Mean, Standard Deviations, and Coefficients of Variation (%) for Cycle Threshold Values: Estimated From Valid Samples of Positive Panel Members**

			Standard Deviation [SD] and Percent Coefficient of Variation [CV(%)]											
			Within-Run		Between-Run		Between-Day		Between-Operator		Between-Site/ Instrument		Total	
Panel Member	n[1]/ N	Mean Ct	SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%
PCR Media/Urine														
1 X LOD CT, Negative NG	180/180	37.1	0.54	1.5%	0.00	0.0%	0.23	0.6%	0.13	0.4%	0.22	0.6%	0.64	1.7%
3 X LOD CT, Negative NG	180/180	35.7	0.38	1.1%	0.18	0.5%	0.15	0.4%	0.00	0.0%	0.21	0.6%	0.50	1.4%
PCR Media/Swab														
1 X LOD CT, Negative NG	174/180	36.9	0.82	2.2%	0.17	0.5%	0.00	0.0%	0.00	0.0%	0.00	0.0%	0.84	2.3%
3 X LOD CT, Negative NG	180/180	36.1	0.42	1.2%	0.24	0.7%	0.14	0.4%	0.14	0.4%	0.00	0.0%	0.53	1.5%
PreservCyt Solution														
1 X LOD CT, Negative NG	177/179	35.2	0.88	2.5%	0.00	0.0%	0.28	0.8%	0.00	0.0%	0.00	0.0%	0.93	2.6%
3 X LOD CT, Negative NG	180/180	33.8	0.68	2.0%	0.03	0.1%	0.18	0.5%	0.15	0.4%	0.00	0.0%	0.72	2.1%

[1] n is the number of positive tests, which contribute Ct values to the analysis. N is the total number of valid tests for the panel member.

***C. trachomatis*: Percent Agreement by Panel Member Overall and for Site/Instrument, Operator, and Day - PCR Media/Urine**

Panel Member	Ct SD	Ct CV %	Percent Agreement*											
			Overall			Site / Instrument			Operator			Day		
1 X LOD CT, Negative NG	0.64	1.7	1	100.0	180/180	1	100.0	60/60	1	100.0	30/30	1	100.0	36/36
						2	100.0	60/60	2	100.0	30/30	2	100.0	36/36
						3	100.0	60/60	3	100.0	30/30	3	100.0	36/36
									4	100.0	30/30	4	100.0	36/36
									5	100.0	30/30	5	100.0	36/36
									6	100.0	30/30			
3 X LOD CT, Negative NG	0.50	1.4	1	100.0	180/180	1	100.0	60/60	1	100.0	30/30	1	100.0	36/36
						2	100.0	60/60	2	100.0	30/30	2	100.0	36/36
						3	100.0	60/60	3	100.0	30/30	3	100.0	36/36
									4	100.0	30/30	4	100.0	36/36
									5	100.0	30/30	5	100.0	36/36
									6	100.0	30/30			
Negative CT, 1 X LOD NG	n/a	n/a	1	100.0	180/180	1	100.0	60/60	1	100.0	30/30	1	100.0	36/36
						2	100.0	60/60	2	100.0	30/30	2	100.0	36/36

Panel Member	Ct SD	Ct CV %	Percent Agreement*											
			Overall			Site / Instrument			Operator			Day		
						3	100.0	60/60	3	100.0	30/30	3	100.0	36/36
									4	100.0	30/30	4	100.0	36/36
									5	100.0	30/30	5	100.0	36/36
									6	100.0	30/30			
	n/a	n/a	1	100.0	179/179	1	100.0	60/60	1	100.0	30/30	1	100.0	36/36
						2	100.0	59/59	2	100.0	30/30	2	100.0	36/36
Negative CT, 3 X LOD NG						3	100.0	60/60	3	100.0	30/30	3	100.0	35/35
									4	100.0	29/29	4	100.0	36/36
									5	100.0	30/30	5	100.0	36/36
									6	100.0	30/30			
	n/a	n/a	1	100.0	180/180	1	100.0	60/60	1	100.0	30/30	1	100.0	36/36
						2	100.0	60/60	2	100.0	30/30	2	100.0	36/36
Negative CT, Negative NG						3	100.0	60/60	3	100.0	30/30	3	100.0	36/36
									4	100.0	30/30	4	100.0	36/36
									5	100.0	30/30	5	100.0	36/36
									6	100.0	30/30			

* For negative samples, percent agreement = (number of negative results/total valid results) x 100; for positive samples, percent agreement = (number of positive results/total valid results) x 100.

Ct = cycle threshold; SD = standard deviation; CV = coefficient of variation; LOD = limit of detection; n/a = not applicable; CT = C. trachomatis; NG = N. gonorrhoeae; n/a = not applicable.

C. trachomatis: Percent Agreement by Panel Member Overall and for Site/Instrument, Operator, and Day - PCR Media/Swab

Panel Member	Ct SD	Ct CV %	Percent Agreement *											
			Overall			Site / Instrument			Operator			Day		
	0.84	2.3	1	96.7	174/180	1	98.3	59/60	1	96.7	29/30	1	100.0	36/36
						2	95.0	57/60	2	100.0	30/30	2	97.2	35/36
1 X LOD CT, Negative NG						3	96.7	58/60	3	96.7	29/30	3	97.2	35/36
									4	93.3	28/30	4	94.4	34/36
									5	96.7	29/30	5	94.4	34/36
									6	96.7	29/30			
	0.53	1.5	1	100.0	180/180	1	100.0	60/60	1	100.0	30/30	1	100.0	36/36
						2	100.0	60/60	2	100.0	30/30	2	100.0	36/36
3 X LOD CT, Negative NG						3	100.0	60/60	3	100.0	30/30	3	100.0	36/36
									4	100.0	30/30	4	100.0	36/36
									5	100.0	30/30	5	100.0	36/36
									6	100.0	30/30			
	n/a	n/a	1	100.0	180/180	1	100.0	60/60	1	100.0	30/30	1	100.0	36/36
						2	100.0	60/60	2	100.0	30/30	2	100.0	36/36
Negative CT, 1 X LOD NG						3	100.0	60/60	3	100.0	30/30	3	100.0	36/36
									4	100.0	30/30	4	100.0	36/36
									5	100.0	30/30	5	100.0	36/36
									6	100.0	30/30			
	n/a	n/a	1	100.0	180/180	1	100.0	60/60	1	100.0	30/30	1	100.0	36/36
Negative CT, 3 X LOD NG						2	100.0	60/60	2	100.0	30/30	2	100.0	36/36
						3	100.0	60/60	3	100.0	30/30	3	100.0	36/36

Panel Member	Ct SD	Ct CV %	Percent Agreement *												
			Overall			Site / Instrument			Operator			Day		
									4	100.0	30/30	4	100.0	36/36
									5	100.0	30/30	5	100.0	36/36
									6	100.0	30/30			
	n/a	n/a	1	99.4	178/179	1	100.0	60/60	1	100.0	30/30	1	100.0	36/36
						2	100.0	59/59	2	100.0	30/30	2	100.0	35/35
Negative CT, Negative NG						3	98.3	59/60	3	100.0	29/29	3	97.2	35/36
									4	100.0	30/30	4	100.0	36/36
									5	96.7	29/30	5	100.0	36/36
									6	100.0	30/30			

* For negative samples, percent agreement = (number of negative results/total valid results) x 100; for positive samples, percent agreement = (number of positive results/total valid results) x 100.
Ct = cycle threshold; SD = standard deviation; CV = coefficient of variation; LOD = limit of detection; n/a = not applicable; CT = C. trachomatis; NG = N. gonorrhoeae; n/a = not applicable.

C. trachomatis: Percent Agreement by Panel Member Overall and for Site/Instrument, Operator, and Day - PreservCyt

Panel Member	Ct SD	Ct CV %	Percent Agreement *												
			Overall			Site / Instrument			Operator			Day		
	0.93	2.6				1	98.3	59/60	1	96.7	29/30	1	100.0	36/36
			2	98.9	177/179	2	98.3	58/59	2	100.0	30/30	2	100.0	36/36
1 X LOD CT, Negative NG						3	100.0	60/60	3	96.7	29/30	3	97.2	35/36
									4	100.0	29/29	4	100.0	35/35
									5	100.0	30/30	5	97.2	35/36
									6	100.0	30/30			
	0.72	2.1				1	100.0	60/60	1	100.0	30/30	1	100.0	36/36
			2	100.0	180/180	2	100.0	60/60	2	100.0	30/30	2	100.0	36/36
3 X LOD CT, Negative NG						3	100.0	60/60	3	100.0	30/30	3	100.0	36/36
									4	100.0	30/30	4	100.0	36/36
									5	100.0	30/30	5	100.0	36/36
									6	100.0	30/30			
	n/a	n/a				1	100.0	60/60	1	100.0	30/30	1	100.0	35/35
			2	100.0	179/179	2	100.0	60/60	2	100.0	30/30	2	100.0	36/36
Negative CT, 1 X LOD NG						3	100.0	59/59	3	100.0	30/30	3	100.0	36/36
									4	100.0	30/30	4	100.0	36/36
									5	100.0	29/29	5	100.0	36/36
									6	100.0	30/30			
	n/a	n/a				1	100.0	60/60	1	100.0	30/30	1	100.0	36/36
			2	100.0	180/180	2	100.0	60/60	2	100.0	30/30	2	100.0	36/36
Negative CT, 3 X LOD NG						3	100.0	60/60	3	100.0	30/30	3	100.0	36/36
									4	100.0	30/30	4	100.0	36/36
									5	100.0	30/30	5	100.0	36/36
									6	100.0	30/30			
	n/a	n/a				1	100.0	60/60	1	100.0	30/30	1	100.0	36/36
			2	100.0	180/180	2	100.0	60/60	2	100.0	30/30	2	100.0	36/36
Negative CT, Negative NG						3	100.0	60/60	3	100.0	30/30	3	100.0	36/36
									4	100.0	30/30	4	100.0	36/36

Panel Member	Ct SD	Ct CV %	Percent Agreement *												
			Overall			Site / Instrument			Operator			Day			
									5	100.0	30/30	5	100.0	36/36	
									6	100.0	30/30				

* For negative samples, percent agreement = (number of negative results/total valid results) x 100; for positive samples, percent agreement = (number of positive results/total valid results) x 100.
Ct = cycle threshold; SD = standard deviation; CV = coefficient of variation; LOD = limit of detection; CT = C. trachomatis; NG = N. gonorrhoeae; n/a = not applicable.

Reproducibility Results for NG:

Testing of samples containing NG concentrations at 3X LOD resulted in 100% positive percent agreement for samples prepared in vaginal swab and PreservCyt matrices and 99.4% agreement for samples prepare in a urine matrix. Samples containing NG concentrations at approximately 1X LOD demonstrated 95%, 89.4%, and 99.4% positive percent agreement respectively for samples prepared in urine, vaginal swab, and PreservCyt matrices. Analysis of variance components of Ct values for NG-positive panel members demonstrated overall CV's ranging from 1.6% to 2.6% for all three matrices. Detailed results from testing are presented in the following tables:

***N. gonorrhoeae*: Overall Mean, Standard Deviations, and Coefficients of Variation (%) for Cycle Threshold: Estimated from Valid Samples of Positive Sample Type Panel Members**

			Standard Deviation [SD] and Percent Coefficient of Variation [CV(%)]											
			Within-Run		Between-Run		Between-Day		Between-Operator		Between-Site/ Instrument		Total	
Panel Member	n[1]/ N	Mean Ct	SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%
PCR Media/Urine														
Negative CT, 1 X LOD NG	171/180	38.00	0.58	1.5%	0.26	0.7%	0.00	0.0%	0.17	0.5%	0.16	0.4%	0.67	1.8%
Negative CT, 3 X LOD NG	178/179	36.93	0.52	1.4%	0.18	0.5%	0.17	0.5%	0.02	0.1%	0.26	0.7%	0.63	1.7%
PCR Media/Swab														
Negative CT, 1 X LOD NG	161/180	37.97	0.58	1.5%	0.24	0.6%	0.05	0.1%	0.27	0.7%	0.00	0.0%	0.68	1.8%
Negative CT, 3 X LOD NG	180/180	37.31	0.56	1.5%	0.12	0.3%	0.00	0.0%	0.08	0.2%	0.15	0.4%	0.60	1.6%
PreservCyt Solution														
Negative CT, 1 X LOD NG	178/179	35.22	0.92	2.6%	0.00	0.0%	0.00	0.0%	0.00	0.0%	0.00	0.0%	0.92	2.6%

			Standard Deviation [SD] and Percent Coefficient of Variation [CV(%)]											
			Within-Run		Between-Run		Between-Day		Between-Operator		Between-Site/ Instrument		Total	
Panel Member	n[1]/ N	Mean Ct	SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%
Negative CT, 3 X LOD NG	180/180	33.72	0.70	2.1%	0.19	0.6%	0.00	0.0%	0.21	0.6%	0.10	0.3%	0.76	2.3%

[1] n is the number of positive tests, which contribute Ct values to the analysis. N is the total number of valid tests for the panel member.

***N. gonorrhoeae*: Percent Agreement by Panel Member Overall and for Site/Instrument, Operator, and Day - PCR Media/Urine**

Panel Member	Ct SD	Ct CV %	Percent Agreement *											
			Overall			Site / Instrument			Operator			Day		
1 X LOD CT, Negative NG	n/a	n/a	1	100.0	180/180	1	100.0	60/60	1	100.0	30/30	1	100.0	36/36
						2	100.0	60/60	2	100.0	30/30	2	100.0	36/36
						3	100.0	60/60	3	100.0	30/30	3	100.0	36/36
									4	100.0	30/30	4	100.0	36/36
									5	100.0	30/30	5	100.0	36/36
									6	100.0	30/30			
3 X LOD CT, Negative NG	n/a	n/a	1	100.0	180/180	1	100.0	60/60	1	100.0	30/30	1	100.0	36/36
						2	100.0	60/60	2	100.0	30/30	2	100.0	36/36
						3	100.0	60/60	3	100.0	30/30	3	100.0	36/36
									4	100.0	30/30	4	100.0	36/36
									5	100.0	30/30	5	100.0	36/36
									6	100.0	30/30			
Negative CT, 1 X LOD NG	0.67	1.8	1	95.0	171/180	1	96.7	58/60	1	96.7	29/30	1	91.7	33/36
						2	96.7	58/60	2	96.7	29/30	2	94.4	34/36
						3	91.7	55/60	3	96.7	29/30	3	100.0	36/36
									4	96.7	29/30	4	91.7	33/36
									5	96.7	29/30	5	97.2	35/36
									6	86.7	26/30			
Negative CT, 3 X LOD NG	0.63	1.7	1	99.4	178/179	1	100.0	60/60	1	100.0	30/30	1	100.0	36/36
						2	100.0	59/59	2	100.0	30/30	2	100.0	36/36
						3	98.3	59/60	3	100.0	30/30	3	100.0	35/35
									4	100.0	29/29	4	100.0	36/36

Panel Member	Ct SD	Ct CV %	Percent Agreement *											
			Overall			Site / Instrument			Operator			Day		
									5	100.0	30/30	5	97.2	35/36
									6	96.7	29/30			
Negative CT, Negative NG	n/a	n/a	1	100.0	180/180	1	100.0	60/60	1	100.0	30/30	1	100.0	36/36
						2	100.0	60/60	2	100.0	30/30	2	100.0	36/36
						3	100.0	60/60	3	100.0	30/30	3	100.0	36/36
									4	100.0	30/30	4	100.0	36/36
									5	100.0	30/30	5	100.0	36/36
									6	100.0	30/30			

*For negative samples, percent agreement = (number of negative results/total valid results) x100; for positive samples, percent agreement = (number of positive results/total valid results) x 100.
Ct = cycle threshold; SD = standard deviation; CV = coefficient of variation; LOD = limit of detection; CT = C. trachomatis; NG = N. gonorrhoeae; n/a = not applicable.

N. gonorrhoeae: Percent Agreement by Panel Member Overall and for Site/Instrument, Operator, and Day - PCR Media/Swab

Panel Member	Ct SD	Ct CV %	Percent Agreement *											
			Overall			Site / Instrument			Operator			Day		
1 X LOD CT, Negative NG	n/a	n/a	1	100.0	180/180	1	100.0	60/60	1	100.0	30/30	1	100.0	36/36
						2	100.0	60/60	2	100.0	30/30	2	100.0	36/36
						3	100.0	60/60	3	100.0	30/30	3	100.0	36/36
									4	100.0	30/30	4	100.0	36/36
									5	100.0	30/30	5	100.0	36/36
									6	100.0	30/30			
3 X LOD CT, Negative NG	n/a	n/a	1	100.0	180/180	1	100.0	60/60	1	100.0	30/30	1	100.0	36/36
						2	100.0	60/60	2	100.0	30/30	2	100.0	36/36
						3	100.0	60/60	3	100.0	30/30	3	100.0	36/36
									4	100.0	30/30	4	100.0	36/36
									5	100.0	30/30	5	100.0	36/36
									6	100.0	30/30			
Negative CT, 1 X LOD NG	0.68	1.8	1	89.4	161/180	1	91.7	55/60	1	93.3	28/30	1	91.7	33/36
						2	85.0	51/60	2	90.0	27/30	2	94.4	34/36
						3	91.7	55/60	3	73.3	22/30	3	88.9	32/36
									4	96.7	29/30	4	86.1	31/36
									5	93.3	28/30	5	86.1	31/36
									6	90.0	27/30			
Negative CT,	0.60	1.6	1	100.0	180/180	1	100.0	60/60	1	100.0	30/30	1	100.0	36/36
						2	100.0	60/60	2	100.0	30/30	2	100.0	36/36

Panel Member	Ct SD	Ct CV %	Percent Agreement *											
			Overall			Site / Instrument			Operator			Day		
3 X LOD NG						3	100.0	60/60	3	100.0	30/30	3	100.0	36/36
									4	100.0	30/30	4	100.0	36/36
									5	100.0	30/30	5	100.0	36/36
									6	100.0	30/30			
Negative CT, Negative NG	n/a	n/a	1	100.0	179/179	1	100.0	60/60	1	100.0	30/30	1	100.0	36/36
						2	100.0	59/59	2	100.0	30/30	2	100.0	35/35
						3	100.0	60/60	3	100.0	29/29	3	100.0	36/36
									4	100.0	30/30	4	100.0	36/36
									5	100.0	30/30	5	100.0	36/36
									6	100.0	30/30			

* For negative samples, percent agreement = (number of negative results/total valid results) x 100; for positive samples, percent agreement = (number of positive results/total valid results) x 100.
Ct = cycle threshold; SD = standard deviation; CV = coefficient of variation; LOD = limit of detection; CT = C. trachomatis; NG = N. gonorrhoeae; n/a = not applicable.

N. gonorrhoeae: Percent Agreement by Panel Member Overall and for Site/Instrument, Operator, and Day - PreservCyt

Panel Member	Ct SD	Ct CV %	Percent Agreement *											
			Overall			Site / Instrument			Operator			Day		
1 X LOD CT, Negative NG	n/a	n/a				1	100.0	60/60	1	100.0	30/30	1	100.0	36/36
			2	100.0	179/179	2	100.0	59/59	2	100.0	30/30	2	100.0	36/36
						3	100.0	60/60	3	100.0	30/30	3	100.0	36/36
									4	100.0	29/29	4	100.0	35/35
									5	100.0	30/30	5	100.0	36/36
									6	100.0	30/30			
3 X LOD CT, Negative NG	n/a	n/a				1	100.0	60/60	1	100.0	30/30	1	100.0	36/36
			2	100.0	180/180	2	100.0	60/60	2	100.0	30/30	2	100.0	36/36
						3	100.0	60/60	3	100.0	30/30	3	100.0	36/36
									4	100.0	30/30	4	100.0	36/36
									5	100.0	30/30	5	100.0	36/36
									6	100.0	30/30			
Negative CT, 1 X LOD NG	0.92	2.6				1	100.0	60/60	1	100.0	30/30	1	100.0	35/35
			2	99.4	178/179	2	98.3	59/60	2	100.0	30/30	2	97.2	35/36
						3	100.0	59/59	3	96.7	29/30	3	100.0	36/36
									4	100.0	30/30	4	100.0	36/36
									5	100.0	29/29	5	100.0	36/36
									6	100.0	30/30			

Panel Member	Ct SD	Ct CV %	Percent Agreement *											
			Overall			Site / Instrument			Operator			Day		
Negative CT, 3 X LOD NG	0.76	2.3				1	100.0	60/60	1	100.0	30/30	1	100.0	36/36
			2	100.0	180/180	2	100.0	60/60	2	100.0	30/30	2	100.0	36/36
						3	100.0	60/60	3	100.0	30/30	3	100.0	36/36
									4	100.0	30/30	4	100.0	36/36
									5	100.0	30/30	5	100.0	36/36
									6	100.0	30/30			
Negative CT, Negative NG	n/a	n/a				1	100.0	60/60	1	100.0	30/30	1	100.0	36/36
			2	100.0	180/180	2	100.0	60/60	2	100.0	30/30	2	100.0	36/36
						3	100.0	60/60	3	100.0	30/30	3	100.0	36/36
									4	100.0	30/30	4	100.0	36/36
									5	100.0	30/30	5	100.0	36/36
									6	100.0	30/30			

* For negative samples, percent agreement = (number of negative results/total valid results) x 100; for positive samples, percent agreement = (number of positive results/total valid results) x 100.
Ct = cycle threshold; SD = standard deviation; CV = coefficient of variation; LOD = limit of detection;
CT = *C. trachomatis*; NG = *N. gonorrhoeae*; n/a = not applicable.

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

Controls:

One set of **cobas** CT/NG v2.0 Test Positive and Negative Controls are required to be included in each run. Valid results must be obtained for both the Positive and Negative Controls for the **cobas** 4800 Software in order for run results to be displayed. The CT/NG positive (+) Control contains non-infectious DNA plasmids of both CT and NG sequences and is used as a run control to monitor the target capture, amplification, and detection steps of the test. The CT/NG Negative (-) Control contains buffer with no nucleic acid.

The **cobas** CT/NG v2.0 Test includes a CT/NG Internal Control that is added to each specimen and control during specimen processing on the **cobas** x instrument. The Internal Control is a combination of two non-infectious recombinant plasmid DNAs, each with primer binding regions identical to those of either the CT or the NG genomic target sequences. The Internal Control monitors test specimens for the presence of PCR inhibitors. The Internal Control is also required for validation of the run controls.

Specimen Stability:

Specimen Stability was evaluated for ambient temperature storage and shipping for the following four specimen types:

- Urine stabilized in **cobas** PCR Media (male and female urine pools)
- Endocervical swab specimens collected in **cobas** PCR Media
- Vaginal swab specimens collected in **cobas** PCR Media
- Cervical specimens collected in PreservCyt solution (primary and secondary containers)

Samples were prepared for each specimen matrix by combining leftover negative specimens into multiple unique pools. CT and NG cultures were spiked into each pooled matrix to approximately 3 X LoD. The study included testing of matrix only samples as well. Testing was performed on the day of sample preparation and at several time points during the storage time period. Study results demonstrated that positive samples remain positive for a minimum of 12 months when stored at 32ºC, with no significant change in Ct values. In addition, matrix-only samples yielded the expectant negative result for the same storage conditions. For PreservCyt matrices that are transferred to a secondary container, study results support the claim of up to four week storage at 30ºC.

Additional assessment of specimen stability was performed for storage of specimens at 2-8ºC. A minimum of 54 positive and 20 negative previously tested archived specimens were tested for each specimen type after long term storage at 2-8ºC. Positive specimens were chosen based on the Ct values obtained when initially tested with the majority being low positives (Ct values above 35). Testing results demonstrated that each specimen type is stable for up to 12 months at 2-8ºC.

In summary, specimen stability study results support the claimed storage conditions.

Processed Specimen Stability

Processed specimen stability was evaluated for each of the four specimen types described above for both ambient air (up to 32ºC) and refrigerated storage conditions (2-8ºC). For this study, 12 specimens for each matrix were spiked with CT and NG cultures at approximately 3X the LoD. Immediately after the completion of sample preparation, extraction plates containing the processed specimens (eluates) were sealed and stored at 2-8ºC for up to eight days or 37ºC for 24 hours. After storage, eluates for each sample were tested using the CT/NG PCR-only workflow. All negative samples and positive samples yielded the expected results at T_0. All positive samples were positive after storage at eight days with no significant change in Ct values. One negative vaginal swab sample gave a positive result at day eight and again upon retesting. It was determined that the most likely cause of this unexpected false positive result was contamination. A new set of positive and negative vaginal swab samples were prepared and tested at T_0 and after seven days and all results were correct. Study results supported processed specimen stability claims of seven days at 2-8ºC and 24 hours at ambient temperature.

d. Detection limit:

The analytical sensitivity (Limit of Detection or LOD) for the **cobas** CT/NG v2.0 Test was determined by testing dilutions of quantified CT (serovars D and I) and NG (isolates

2948 and 6693) cultures into the following matrices:

- Pooled negative endocervical swab specimens in **cobas** PCR Media
- Pooled negative vaginal swab specimens in **cobas** PCR Media
- Pooled negative male and female urine specimens in **cobas** PCR Media
- Pooled negative cervical specimen in PreservCyt Solution

All CT serovars and NG strains were tested at five concentration levels (60 replicates for each level). In addition, 24 replicates of a negative sample were tested. Replicates for each level were analyzed using the **cobas** CT/NG v2.0 Test across three lots of reagents with the claimed LOD as the target concentration which can be detected in ≥95% of tested replicates.
The LOD for the CT serovar D and I cultures and NG strains 2948 and 6693 for each matrix are shown in the following table:

cobas CT/NG v2.0 Test Limit of Detection

Specimen Types	***C. trachomatis***		***N. gonorrhoeae***	
	Serovar D	**Serovar I**	**Strain 2948**	**Strain 6693**
	LOD (EB/mL)	LOD (EB/mL)	LOD (CFU/mL)	LOD (CFU/mL)
Endocervical Swabs	200	100	2.0	2.0
Vaginal Swabs	300	70	3.0	1.5
Male Urine*	40	20	0.2	0.6
Female Urine*	40	10	0.2	0.4
PreservCyt Cervical	200	50	1.0	1.0

EB = Elementary Body; CFU = Colony Forming Units

*The stated LOD for male and female urine are for urine samples diluted in cobas PCR media. The LOD for neat urine (before dilution) is twice the LOD for diluted urine.

e. Inclusivity

Inclusivity testing with the **cobas** CT/NG v2.0 Test was performed for 13 additional CT serovars, the Swedish new variant strain (nvCT) and an additional 43 independently isolated strains of NG. Testing was done to demonstrate that these targets can be detected at concentrations near the LOD previously determined for CT serovars D and I and NG strains 2948 and 6693. Samples for this study were prepared by diluting titered CT and NG cultures into pooled negative endocervical swab specimen matrix in **cobas** PCR Media, pooled negative vaginal swab specimen matrix in **cobas** PCR Media, pooled negative urine specimen matrix plus **cobas** PCR Media and pooled negative cervical specimen in PreservCyt Solution. A minimum of 20 replicates were tested for each strain, dilution, and matrix using one lot of **cobas** CT/NG v2.0 Test reagents. Results are shown in the tables below. For NG, all strains with identical results are presented as a group, shown in the columns labeled "Numbers of NG Strains".

Summary of CT Serovars/Variant Inclusivity Verification Results

Serovar Type or Variant	Endocervical Swabs		Vaginal Swabs		Urine		PreservCyt	
	EB/mL	% Pos	EB/mL	% Pos	EB/mL	% Pos	EB/mL	% Pos
A	100	100	150	100	20	100	100	100
B	100	100	150	100	20	100	100	100
Ba	100	100	150	100	20	100	100	100
C	100	100	150	100	20	100	100	100
E	100	100	150	100	20	100	100	100
F	100	100	150	100	20	100	100	100
G	100	95	150	100	20	100	100	100
H	100	95	150	100	20	100	100	100
J	100	100	150	100	20	100	100	100
K	100	100	150	100	20	100	100	100
LGV Type 1	100	100	150	100	20	100	100	100
LGV Type 2	100	100	150	100	20	100	100	100
LGV Type 3	100	100	150	100	20	100	100	100
nvCT	300	100	150	95	60	100	100	100

Summary of NG Strains Inclusivity Verification Results

Numbers of NG Strains	Inclusivity Results for **Endocervical Specimens**	
	CFU/mL	% Hit Rate
39	3.0	≥95
4	10.0	100
Total = 43		
Numbers of NG Strains	Inclusivity Results for **Vaginal Specimens**	
	CFU/mL	% Hit Rate
42	4.5	≥95
1	10.0	100
Total = 43		
Numbers of NG Strains	Inclusivity Results for **Urine**	
	CFU/mL	% Hit Rate
34	0.3	≥95

f. Analytical specificity:

Because there is no change in the assay reagents for the **cobas** CT/NG v2.0 Test as compared to the cleared **cobas** CT/NG Test (K110923), it was concluded that the data for the analytical specificity testing from the first clearance could be used to support the performance of the v2.0 test. In this study, a panel of 184 bacteria, fungi, and

viruses, including those commonly found in the female urogenital tract, as well as representatives of *N. cineria, N. flava N. lactamica, N. perflava*, *N. subflava,* and other phylogenetically unrelated organisms, were tested to assess potential cross-reactivity (see table immediately following this section). Samples were spiked at concentrations of 1 x 10^6 Units*/mL or higher for the majority of organisms tested. The second table below lists selected organisms that were evaluated at lower concentrations. Organisms were spiked into pooled negative urine in **cobas** PCR Media, pooled negative vaginal matrix in **cobas**® PCR Media, and pooled negative PreservCyt specimen matrices. No false positive results or interference of the internal control (IC) signal were observed for any of the organisms and matrices evaluated.

*Definition of Units/mL: All bacteria were quantified as Colony Forming Units (CFU) except *Chlamydophila pneumoniae* was quantified as Inclusion Forming Units (IFU). *Treponema pallidum* and HBV were quantified as DNA copies. Adenovirus was quantified as Plaque Forming Units (PFU). CMV, EBV, HSV-1 and HSV-2 were quantified as Viral Particles (VP). HCV and HIV-1 were quantified in International Units (IU). *Trichomonas vaginalis*, HPV16 and HPV18 were quantified as cells/mL.

Microorganisms Tested for Analytical Specificity

Achromobacter xerosis	*Helicobacter pylori*	*Neisseria sicca*
Acinetobacter calcoaceticus	Hepatitis B virus (HBV)	*Neisseria subflava*
Acinetobacter lwoffi	Hepatitis C virus (HCV)	*Neisseria subflava 6458*
Acinetobacter sp. genospecies 3	Human immunodeficiency virus	*Neisseria subflava 6617*
Actinomyces israelii	Human papillomavirus type 16 (CaSki cells)	*Neisseria subflava 6618*
Actinomyces pyogenes	Human papillomavirus type 18 (HeLa cells)	*Neisseria subflava 7441*
Adenovirus	Herpes Simplex Virus (HSV-1)	*Neisseria subflava 7452*
Aerococcus viridans	Herpes Simplex Virus (HSV-2)	*Neisseria weaverii*
Aeromonas hydrophila	*Kingella dentrificans*	*Pantoea agglomerans*
Alcaligenes faecalis	*Kingella kingae*	*Paracoccus denitrificans*
Bacillus subtilis	*Klebsiella oxytoca*	*Pasteurella maltocida*
Bacillus thuringiensis	*Klebsiella pneumoniae ss ozaenae*	*Pediococcus acidilactica*
Bacteroides caccae	*Lactobacillus acidophillus*	*Peptostreptococcus anaerobius*
Bacteroides fragilis	*Lactobacillus brevis*	*Peptostreptococcus asacharolyticus*
Bacteroides ureolyticus	*Lactobacillus crispatus*	*Peptostreptococcus magnus*
Bifidobacterium adolescentis	*Lactobacillus delbrueckii subsp. lactis*	*Plesiomonas shigelloides*
Bifidobacterium breve	*Lactobacillus jensenii*	*Prevotella bivia*
Bifidobacterium longum	*Lactobacillus lactis*	*Prevotella corporis*
Branhamella catarrhalis	*Lactobacillus oris*	*Prevotella intermedia*
Brevibacterium linens	*Lactobacillus parabuchnerri*	*Propionibacterium acnes*
Campylobacter gracilis	*Lactobacillus vaginalis*	*Proteus mirabilis*
Campylobacter jejuni	*Lactococcus lactis cremoris*	*Proteus vulgaris*
Candida albicans	*Legionella bozemnii*	*Providencia stuartii*
Candida glabrata	*Legionella pneumophila*	*Pseudomonas aeruginosa*
Candida guilliermondi	*Listeria monocytogenes*	*Pseudomonas fluorescens*

Candida krusei	*Micrococcus luteus*	*Pseudomonas putida*
Candida parapsilosis	*Mobiluncus curtisii subsp. curtisii*	*Rahnella aquatilis*
Candida tropicalis	*Mobiluncus curtisii subsp. holmesii*	*Rhizobium radiobacter*
Chlamydophila pneumoniae	*Mobiluncus mulieris*	*Rhodospirillum rubrum*
Chromobacter violaceum	*Moraxella catarrhalis*	*Ruminococcus productus*
Chryseobacterium meningosepticum	*Moraxella lacunata*	*Saccharomyces cerevisiae*
Citrobacter braakii	*Moraxella osloensis*	*Salmonella* Choleraesuis
Citrobacter freundii	*Morganella morganii*	*Salmonella* Minnesota
Clostridium innocuum	*Mycobacterium avium*	*Salmonella* typhimurium
Clostridium perfringens	*Mycobacterium gordonae*	*Serratia denitrificans*
Clostridium sporogenes	*Mycobacterium smegmatis*	*Serratia marcescens*
Corynebacterium genitalium	*Mycoplasma genitalium*	*Staphylococcus aureus*
Corynebacterium renale	*Mycoplasma hominis*	*Staphylococcus epidermidis*
Corynebacterium xerosis	*Mycoplasma pneumoniae*	*Staphylococcus saprophyticus*
Cryptococcus neoformans	*Neisseria cinerea 832*	*Streptococcus agalactiae*
Cytomegalovirus	*Neisseria cinerea 3306*	*Streptococcus anginosus*
Deinococcus radiodurans	*Neisseria cinerea 3307*	*Streptococcus bovis*
Deinococcus radiopugnans	*Neisseria cinerea 3308*	*Streptococcus dysgalactiae*
Derxia gummosa	*Neisseria cinerea 6317*	*Streptococcus equinis*
Edwardsiella tarda	*Neisseria dentrificans*	*Streptococcus mitis*
Eikenella corrodens	*Neisseria elongata subsp. niroreducans*	*Streptococcus mutans*
Enterobacter aerogenes	*Neisseria flava*	*Streptococcus pneumoniae*
Enterobacter cloacae	*Neisseria flavescens*	*Streptococcus pyogenes*
Enterococcus avium	*Neisseria kochi*	*Streptococcus salivarius*
Enterococcus faecalis	*Neisseria lactamica*	*Streptococcus sanguis*
Enterococcus faecium	*Neisseria meningitidis 135*	*Streptomyces griseinus*
Epstein Barr Virus	*Neisseria meningitidis Serogroup A*	*Treponema pallidum*
Erwinia herbicola	*Neisseria meningitidis Serogroup B*	*Trichomonas vaginalis*
Erysipelothrix rhusiopathiae	*Neisseria meningitidis Serogroup C*	*Ureaplasma urealyticum*
Escherichia coli	*Neisseria meningitidis Serogroup D*	*Veillonela parvula*
Ewingella americana	*Neisseria meningitidesSerogroup Y*	*Vibrio parahaemolyticus*
Flavobacterium meningosepticum	*Neisseria mucosa*	*Weissella paramesenteroides*
Fusobacterium nucleatum	*Neisseria perflava 837*	*Yersinia enterocolitica*
Gardnerella vaginalis	*Neisseria perflava 911*	
Gemella haemolysans	*Neisseria perflava 6339*	
Gemella morbillorum	*Neisseria perflava 6340*	

Haemophilus ducreyi	*Neisseria perflava 6341*	
Haemophilus influenzae	*Neisseria polysaccharea*	

Microorganisms Tested for Analytical Specificity at Less than 1 x 10^6 Units/mL

Microorganism Tested	Concentration Tested in Listed Matrix*			
	cobas® PCR Media	**Negative Urine Specimen**	**Negative Vaginal Specimen**	**Negative PreservCyt Specimen**
Adenovirus		$8x10^5$ PFU/mL	$8x10^5$ PFU/mL	$8x10^5$ PFU/mL
Cytomegalovirus (CMV)	$1x10^4$ VP/mL			
Chlamydophila pneumoniae	$1x10^5$ IFU/mL	$1.1x10^4$ IFU/mL	$1.1x10^4$ IFU/mL	$1.1x10^4$ IFU/mL
Gemella morbillorum		4.5×10^4 CFU/mL	4.5×10^4 CFU/mL	4.5×10^4 CFU/mL
Hepatitis C virus (HCV)		5.6×10^4 IU/mL	5.6×10^4 IU/mL	5.6×10^4 IU/mL
Human papillomavirus (HPV) type 16 (SiHa cells)		$1x10^4$ cells/mL	$1x10^4$ cells/mL	$1x10^4$ cells/mL
Human papillomavirus (HPV) type 18 (HeLa cells)		$1x10^4$ cells/mL	1x104 cells/mL	1x104 cells/mL
Neisseria cinerea 3307			4x105 CFU/mL	4x105 CFU/mL
Prevotella bivia		9x104 CFU/mL	9x104 CFU/mL	9x104 CFU/mL
Prevotella corporis			1.4x105 CFU/mL	1.4x105 CFU/mL
Treponema pallidum	Not Tested	1x105 copies/mL	1x105 copies/mL	1x105 copies/mL
Trichomonas vaginalis			6.5x105 cells/mL	6.5x105 cells/mL

*Gray cells indicate concentration tested was ≥ 1 x 10^6 Units/mL in that matrix

g. Microbial interference

All organisms tested in the analytical specificity study listed in the table above were also evaluated for potential microbial interference with the detection of CT and/or NG. In this study, samples were prepared with each potentially interfering organism mixed with CT and NG cultures at approximately 3 times their respective LoDs. In addition, CT/NG positive and negative test samples (with no spiking of additional organisms) were used as controls. Study results demonstrated no false negative results and no deterioration in Ct values for either CT or NG analytes in all evaluated matrices.

h. Interference Study

Testing of Exogenous Substances:

Performance of the **cobas** CT/NG v2.0 Test was evaluated in the presence of potentially interfering exogenous substances including over-the counter products and prescription drugs that may be present in clinical specimens. Samples were prepared in pooled negative endocervical swab specimen matrix collected in **cobas** PCR Media, pooled negative vaginal swab specimen matrix collected in cobas® PCR Media, pooled negative urine specimen matrix plus cobas® PCR Media and pooled negative cervical specimen matrix collected in PreservCyt Solution. Positive samples were spiked with each potentially interfering substance and CT and NG cultures at ~3 x LOD for each target. For each substance and matrix evaluated, replicates of ten CT/NG positive and ten CT/NG negative samples were tested. Substances and matrices tested are listed in the following table.

Exogenous Substances

Product	Urine	Endocervical	Vaginal	PreservCyt
Azo Standard, Maximum Strength	X	X	X	X
Clindamycin Phosphate Vaginal Cream	X	X	X	X
Clotrimazole 7	X	X	X	X
CVS tioconazole 1	X	X	X	X
Estrace	X	X	X	X
Gyne-Lotrimin 7	X	X	X	X
KY Jelly	X	X	X	X
KY Silk-E	X	X	X	X
Metronidazole Vaginal Gel	X	X	X	X
Miconazole	X	X	X	X
Monistat 3 Cream	X	X	X	X
Monistat Soothing Care Itch Relief Cream	X	X	X	X
Norforms	X	X	X	X
Premarin	X	X	X	X
Preparation H	NT	X	X	X
Replens	X	X	X	X
Summer's Eve Deodorant Spray	X	NT	NT	NT
Vagicaine Anti-Itch Cream	X	X	X	X
Vagi-Gard (Douche)	X	X	X	X
Vagi-Gard Medicated Cream	X	X	X	X
Vaginal Contraceptive Foam	X	X	X	X
Vagisil Moisturizer	X	X	X	X
Vagisil Satin	X	X	X	X
Yeast Gard	X	X	X	X

X = Tested; NT = Not tested

Results from this study demonstrated that 20 of the 23 exogenous substances tested did not interfere with the performance of the assay for detection of CT and NG. The presence of Metronidazole vaginal gel and Vagisil Satin were found to produce invalid and/or false negative results for either CT and/or NG in urine samples. Replens was found to produce invalid and/or false negative results for either CT and/or NG in both urine and

endocervical swab matrices. Limitations are included in the package insert describing assay interference with metronidazole vaginal gel, Vagisil Satin, and Replens.

Testing of Endogenous Substances:

Performance of the **cobas** CT/NG v2.0 Test was evaluated in the presence of potentially-interfering endogenous substances including whole blood, peripheral blood mononuclear cells (PBMC), and mucus. Samples were prepared in urine, vaginal, endocervical, and PreservCyt matrices. For each substance and matrix evaluated, replicates of ten CT/NG positive and ten CT/NG negative samples were tested. Positive samples were spiked with each potentially interfering substance and CT and NG cultures at ~3 x LOD for each target.

The presence of whole blood lead to false negative results when present in urine samples containing greater than 0.25% blood and in cervical specimens in PreservCyt solution at concentrations greater than 3%. In addition, invalid results were observed in urine samples containing whole blood concentrations greater than 0.25% and in PreservCyt samples containing whole blood at concentrations 5% or higher.

The presence of PBMC resulted in false negative results when present in urine samples containing greater than 1×10^5 cells/mL and in vaginal or endocervical samples containing greater than 1×10^6 cells/mL. Invalid or failed tests occurred in samples containing 1×10^7 PBMC cells/mL presumably due to sample viscosity.

Limitations are included in the package insert describing each endogenous substance that demonstrated interference with the **cobas** CT/NG v2.0 Test.

Potentially interfering endogenous substances and matrices tested are described in the following table. Concentrations in urine samples were determined using total sample volume, including stabilizing media.

Results from Endogenous Interference Testing

	Blood (v/v)		**PBMC (cells/mL)**		**Mucus**	
	Conc. Tested	**Interference Observed**	**Conc. Tested**	**Interference Observed**	**Conc. Tested**	**Interference Observed**
Urine stabilized in **cobas** PCR Media	0, 0.1%, 0.25%, 0.5%, 1%	> 0.25%	0, 1.0E+05, 1.0E+06, 1.0E+07	> 1x 105	NT	NT
Vaginal Specimen collected in **cobas** PCR Media	0, 3%, 5%, 10%	None	0, 1.0E+05, 1.0E+06, 1.0E+07	> 1 x 106	Routine level*	None
Endocervical Specimen collected in **cobas** PCR Media	0, 3%, 5%, 10%	None	0, 1.0E+05, 1.0E+06, 1.0E+07	> 1 x 106	Routine level*	None
Cervical Specimens collected in PreservCyt	0, 3%, 5%, 10%	> 3%	0, 1.0E+05, 1.0E+06,	None	Routine level*	None

Solution			1.0E+07			

NT = Not Tested

*Routine level = Quantity of cervical mucus equivalent to amount normally removed prior to sampling

The following additional endogenous substances were evaluated in samples prepared with pooled negative urine in **cobas** PCR Media. Testing results demonstrated no interference in either positive or negative samples.

Additional Endogenous Interference Testing in Urine Stabilized in cobas PCR Media

Substance Tested	**Levels Tested**	**Interference Observed**
Albumin	0%, 1%, 2%, and 5% (w/v)	None
Glucose	0%, 0.5% and 1% (w/v)	None
Bilirubin	0%, 0.05%, 0.1%, 0.25% and 0.5% (w/v)	None
Acidic Condition	pH 4	None
Basic Condition	pH 9	None

i. Carry over/contamination

CT/NG Workflow: Sample-to-sample cross-contamination and run-to-run carryover studies were performed using the CT/NG Workflow with samples in **cobas** PCR media and urine stabilized in **cobas** PCR Media. Two runs consisting of a checkerboard pattern of high-positive CT and NG samples alternating with negative samples were performed on each of three **cobas** 4800 Systems. Contrived samples were prepared by spiking high concentrations of CT and NG cultures into each matrix such that analyte concentrations would be higher than found in 95% of specimens of infected individuals in the intended use population. Study results for the CT/NG Workflow yielded a sample-to-sample cross contamination rate of 1.07% (6/562) and a run-to-run carry over rate of 0.18% (1/563).

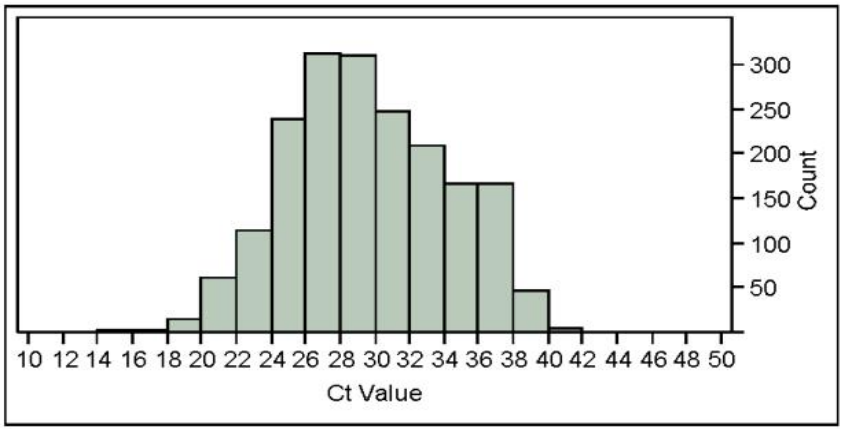
CT/NG Cytology Workflow: Three additional runs were performed on each of three **cobas** 4800 Systems using the CT/NG Cytology Workflow using contrived samples in PreservCyt Solution. Again, specimens were tested in a checkerboard configuration of high-positive CT and NG specimens alternating with negative samples. The CT/NG Cytology Workflow gave a sample-to-sample cross-contamination rate of 1.18% (5/423) and a run-to-run carry over rate of 0.0% (0/280).

ThinPrep 2000 Workflow: The rate of sample-to-sample cross-contamination events during routine processing of PreservCyt specimens on the ThinPrep 2000 liquid cytology processor was performed using alternating CT/NG negative and CT/NG high-positive samples. After cytology processing, the PreservCyt samples were then tested with the **cobas** CT/NG v2.0 Test. False positive results occurred in two of 427 CT/NG negative samples, resulting in a cross-contamination rate of 0.81%.

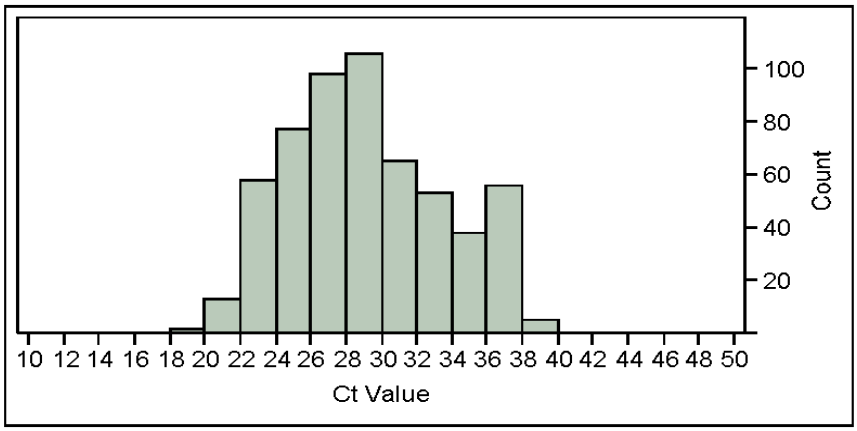
j. Assay cut-off:

The assay cutoff (Ct value = 45) for the **cobas** CT/NG Test (K110923) that was validated for the initial clearance of the assay was also selected for the **cobas** CT/NG v2.0 Test. The chosen cutoff was subsequently validated for the **cobas** CT/NG v2.0 Test by analyzing all results from the pivotal clinical study and selected analytical studies. All specimen types were included in the analysis. The distribution of Ct values for positive specimens was analyzed and it was determined that the chosen cutoff is acceptable due to the evidence of a clear distinction between positive and negative results for all specimen types. The following graphs illustrate the distribution of Ct values from the clinical study.

Ct Distribution of CT Positive Specimens



Ct Distribution of NG Positive Specimens



k. Competitive Inhibition Studies

Panels were prepared by spiking CT and NG cultures into pooled vaginal specimen matrix collected in **cobas** PCR Media, pooled urine matrix stabilized in **cobas** PCR Media and pooled cervical specimen matrix collected in PreservCyt Solution at various

concentration levels to examine the potential for competitive inhibition. Panels were tested in two runs per day over the course of 12 days. Two replicates of each panel member were tested in every run, generating a maximum of 48 test results for each level and CT and NG strain respectively. All CT and NG hit rates were 100% for all panel levels in all sample matrices tested. Competitive inhibition was not seen in any combination of CT and NG levels in any of the three sample matrices tested. Average Ct values for each of the panel levels are summarized in the table below.

Competitive Inhibition Study Results

Panel Level		**CT Mean Ct**	**NG Mean Ct**
CT Level (EB/mL)	**NG Level (CFU/mL)**		
Vaginal Swab Specimen Collected in cobas PCR Media			
Low (300)	High (1.0E+06)	36.6	18.6
High (1.0E+07)	Low (3)	21.6	36.8
High (1.0E+07)	High (1.0E+06)	21.2	18.1
Urine Stabilized in cobas PCR Media			
Low (40)	High (1.0E+05)	36.1	18.4
High (1.0E+07)	Low (0.2)	18.0	36.7
High (1.0E+07)	High (1.0E+05)	18.3	18.0
Cervical Specimen Collected in PreservCyt Solution			
Low (200)	High (1.0E+05)	34.8	18.9
High (1.0E+07)	Low (1)	18.9	34.3
High (1.0E+07)	High (1.0E+05)	18.8	17.7

2. Comparison studies:

   a. Method comparison with predicate devices:

   Not applicable

   b. Matrix comparison:

   Not applicable

3. Clinical studies:

   The clinical performance of the **cobas** CT/NG v2.0 Test was established in two multi-center clinical studies conducted in the United States. One clinical study included testing of archived endocervical specimens, self-collected and clinician collected vaginal specimens and urine specimens from symptomatic and asymptomatic males and females, initially collected prospectively during the clinical evaluation of the **cobas** CT/NG Test (K110923). The current study included testing of all available prospectively collected specimens stored at 2-8ºC since the original study.

   The second clinical study included only asymptomatic female subjects and the following

four prospectively collected and tested fresh specimens: endocervical specimens, clinician-collected vaginal specimens, cervical specimens in PreservCyt Solution, and female urines.

For both clinical studies, specimen collection took place at 18 collection sites in the US, which included family planning and obstetrics/gynecology (OB/GYN) clinics, and sexually transmitted disease clinics. Testing was performed at seven laboratory sites.

Each female subject provided a urine specimen, a self-collected or clinician-collected vaginal swab, a clinician-collected endocervical swab, and a cervical specimen in PreserCyt solution obtained with a spatula/cytobrush or a broom. Aliquots of urine and cervical specimens in PreserCyt as well as a second endocervical swab were placed in the respective transport media for determination of patient infected status (PIS) using two commercially available nucleic acid amplification tests (NAAT).

Each male subject provided a urine specimen in **cobas** PCR media and urine and urethral swab specimens in collection media from two commercially available NAAT assays.

Subjects were classified as symptomatic if they reported symptoms indicative of CT or NG infection, as listed below. Subjects were classified as asymptomatic if they did not report these symptoms.

- Dysuria/pain during urination, coital pain/difficulty/bleeding, discharge, or pelvic pain
- Abnormal vaginal discharge
- Pelvic/uterine/ovarian pain
- Urethral discharge, testicular pain/scrotal pain/swelling

The clinical performance of the **cobas** CT/NG v2.0 Test was evaluated by comparing the results from collected sample types to a pre-specified PIS algorithm as determined by combined results from two commercially available NAATs. The PIS algorithms for female and male patients are shown in the following two tables.

Algorithm for Female Patient Infected Status

NAAT1 Urine/Endocervical	NAAT2 Urine/Endocervical	NAAT2 Cervical Swab in PreservCyt Solution	Patient Infected Status (PIS)
+/+	+/+	+ or -	Infected
+/+	+/- or -/+	+ or -	Infected
+/- or -/+	+/+	+ or -	Infected
+/-	-/+	+ or -	Infected
-/+	+/-	+ or -	Infected
-/+	-/+	+ or -	Infected
+/-	+/-	+	Infected
+/-	+/-	-	**CT:** Infected (Urine) Non-Infected (Swabs)

NAAT1 Urine/Endocervical	NAAT2 Urine/Endocervical	NAAT2 Cervical Swab in PreservCyt Solution	Patient Infected Status (PIS)
			NG: Infected (Urines and Swabs)
+/- or -/+	-/-	+ or -	Non-Infected
+/+	-/-	+ or -	Non-Infected
-/-	+/+	+ or -	Non-Infected
-/-	+/- or -/+	+ or -	Non-Infected
-/-	-/-	+ or -	Non-Infected

Algorithm for Male Patient Infected Status

NAAT 1 Urethra Swab/Urine	NAAT 2 Urethral Swab/Urine	Patient Infected Status (PIS)
+/+	+/+	Infected
+/+	+/- or -/+	Infected
+/- or -/+	+/+	Infected
+/-	-/+	Infected
-/+	+/-	Infected
-/+	-/+	Infected
+/-	+/-	Infected
+/- or -/+	-/-	Non-Infected
+/+	-/-	Non-Infected
-/-	+/+	Non-Infected
-/-	+/- or -/+	Non-Infected
-/-	-/-	Non-Infected

Primary objectives of the study included the evaluation the sensitivity (SENS), specificity (SPEC), positive predictive value (PPV), and negative predictive value (NPV) of the **cobas** CT/NG v2.0 Test for detection of CT or NG using PIS as the reference standard. Results were stratified by gender, sample type, and symptom status. Predictive values were calculated based on sensitivity and specificity with all data combined for a range of hypothetical prevalence values.

Of the 6,045 subjects enrolled (5,306 females and 739 males), ten were excluded from the analyses because they did not meet study entry criteria or because they withdrew consent; 31 were considered non-evaluable and were excluded from all statistical analyses because of errors in specimen collection, transport, storage, unknown PIS for both CT and NG, or invalid **cobas** CT/NG v2.0 Test results after initial testing and/or retesting. Therefore, of 6,035 total eligible subjects enrolled, 6,004 (99.5%) were evaluable for CT and/or NG primary analyses (5,266 females and 738 males). Results obtained from 1,011 prospectively tested specimens from female asymptomatic subjects were analyzed combined with results obtained in the re-testing of archived specimens collected in the previous clinical study (archived specimens collected from 4,255 females and 738 males).

In both clinical studies, there was 1/385 (0.3%) invalid runs and 15/385 (3.9%) failed runs due to instrument errors. Of the 26,283 specimens tested with the **cobas** CT/NG v2.0 Test, 0.28% and 0.23% were initially invalid for CT and NG respectively and 1.38% initially had failed results for both CT and NG. Following retesting up to two more times, 0.10% and 0.13% had final results of invalid for CT and NG respectively and 0.14% had final failed results for both CT and NG.

Clinical Study Results for *Chlamydia trachomatis* (CT)

The following two tables summarize the results from symptomatic and asymptomatic female and male subjects designated as infected or non-infected with CT according to the PIS algorithm. A total of 365 females and 122 males were infected with CT. Symptoms were reported in 44.4% (162/365) of infected and 37.0% (1,814/4,900) of non-infected women. Similarly, symptoms were reported in 57.4% (70/122) of infected and 33.8% (208/616) of non-infected men. Overall, the CT prevalence was 6.9% (365/5,265), 16.5% (122/738), and 8.1% (487/6,003) in women, men, and the entire study population, respectively.

CT: Positive/Negative Analysis for Female Patient Infected Status

Patient Infected Status	NAAT1a		NAAT2a			cobas CT/NG v2.0 Test					Symptom Status		Total
	SW	UR	SW	UR	PC Pre	SW	UR	VG	PC Pre	PC Post	Symp	Asymp	
Infected	+	+	+	+	+	+	+	+	+	+	113	144	257
Infected	+	+	+	+	+	NA	+	+	+	+	3	7	10
Infected	+	-	+	-	+	+	-	+	+	+	3	2	5
Infected	-	+	-	+	-	-	+	-	-	-	2	3	5
Infected	+	+	+	+	+	+	+	+	+	NA	3	1	4
Infected	+	+	+	-	+	+	+	+	+	+	2	2	4
Infected	+	-	+	+	+	+	+	+	+	+	4	0	4
Infected	+	+	+	+	+	+	+	+	-	+	2	1	3
Infected	+	-	+	+	+	+	-	+	+	+	1	2	3
Infected	+	+	+	+	+	+	+	+	-	-	1	1	2
Infected	+	+	+	+	+	+	+	+	NA	NA	1	1	2
Infected	+	+	+	+	+	+	-	+	+	+	1	1	2
Infected	+	+	+	+	+	NA	+	NA	+	+	0	2	2
Infected	+	+	+	+	NA	+	+	+	NA	NA	1	1	2
Infected	+	-	+	-	-	+	-	-	-	-	0	2	2
Infected	-	+	+	+	+	+	+	+	+	+	0	2	2
Infected	-	+	+	+	+	-	+	+	-	-	1	1	2
Infected	-	+	+	+	-	-	+	+	-	-	2	0	2
Infected	-	+	-	+	-	-	+	+	-	-	1	1	2
Infected	+	+	+	+	+	+	+	+	NA	+	0	1	1
Infected	+	+	+	+	+	+	+	NA	+	+	1	0	1
Infected	+	+	+	+	+	+	-	+	+	-	0	1	1
Infected	+	+	+	+	+	+	NA	+	+	+	1	0	1

Patient Infected Status	NAAT1a		NAAT2a			cobas CT/NG v2.0 Test					Symptom Status		Total
	SW	UR	SW	UR	PC Pre	SW	UR	VG	PC Pre	PC Post	Symp	Asymp	
Infected	+	+	+	+	+	-	+	+	+	+	1	0	1
Infected	+	+	+	+	+	-	+	+	+	-	0	1	1
Infected	+	+	+	+	+	-	+	+	-	-	0	1	1
Infected	+	+	+	+	-	+	+	+	-	+	0	1	1
Infected	+	+	+	+	NA	NA	+	+	NA	NA	1	0	1
Infected	+	+	-	+	+	+	+	+	+	+	0	1	1
Infected	+	+	-	+	+	-	+	+	+	+	1	0	1
Infected	+	+	-	+	+	-	+	+	+	-	0	1	1
Infected	+	+	-	+	+	-	+	+	-	-	0	1	1
Infected	+	+	-	+	-	+	+	+	+	NA	1	0	1
Infected	+	+	NA	+	NA	NA	NA	NA	NA	+	0	1	1
Infected	+	+	+	-	+	+	+	+	NA	+	1	0	1
Infected	+	+	+	-	+	+	+	-	+	+	0	1	1
Infected	+	+	+	-	+	+	-	+	+	+	0	1	1
Infected	+	+	+	-	+	-	+	+	-	-	0	1	1
Infected	+	+	+	-	+	NA	+	+	+	+	0	1	1
Infected	+	+	+	-	-	+	+	+	-	-	0	1	1
Infected	+	+	+	-	-	-	+	NA	-	NA	0	1	1
Infected	+	+	+	NA	+	+	+	+	+	+	1	0	1
Infected	+	-	+	+	+	+	+	+	+	-	0	1	1
Infected	+	-	+	+	+	+	+	+	+	NA	1	0	1
Infected	+	-	+	+	+	+	+	-	+	+	0	1	1
Infected	+	-	+	+	+	+	-	-	+	+	1	0	1
Infected	+	-	+	+	+	NA	+	+	+	-	1	0	1
Infected	+	-	+	-	+	+	+	+	+	+	0	1	1
Infected	+	-	+	-	+	+	-	+	+	-	0	1	1
Infected	+	-	+	-	+	+	-	-	+	+	1	0	1
Infected	+	-	+	-	+	-	-	+	-	-	0	1	1
Infected	+	-	+	-	-	+	-	+	+	+	0	1	1
Infected	+	-	+	-	-	-	-	NA	-	-	0	1	1
Infected	+	-	+	-	NA	+	+	+	-	-	0	1	1
Infected	+	-	+	NA	+	+	-	-	-	-	1	0	1
Infected	+	NA	+	NA	+	+	NA	+	NA	NA	1	0	1
Infected	-	+	+	+	+	+	+	+	-	+	1	0	1
Infected	-	+	+	+	+	-	+	+	+	+	1	0	1
Infected	-	+	+	+	+	-	+	+	-	+	0	1	1
Infected	-	+	+	+	+	-	+	-	-	-	1	0	1
Infected	-	+	+	+	-	-	+	+	+	-	0	1	1
Infected	-	+	+	+	-	-	-	+	+	-	1	0	1
Infected	-	+	-	+	+	+	+	+	+	-	1	0	1
Infected	-	+	-	+	-	+	+	+	-	+	0	1	1
Infected	-	+	-	+	-	-	+	+	+	+	1	0	1
Infected	-	+	-	+	-	-	+	+	-	+	1	0	1

Patient Infected Status	NAAT1a		NAAT2a			cobas CT/NG v2.0 Test					Symptom Status		Total
	SW	UR	SW	UR	PC Pre	SW	UR	VG	PC Pre	PC Post	Symp	Asymp	
Infected	-	+	-	+	NA	-	+	-	NA	NA	0	1	1
Infected	-	+	NA	+	-	+	+	+	-	-	0	1	1
Infected	-	+	+	-	-	-	-	+	-	-	0	1	1
Total Infected											**162**	**203**	**365**
Non-Infected	-	-	-	-	-	-	-	-	-	-	1575	2561	4136
Non-Infected	-	-	-	-	-	-	-	-	-	NA	57	178	235
Non-Infected	-	-	-	-	-	NA	-	-	-	-	27	30	57
Non-Infected	-	-	-	-	-	-	-	NA	-	-	26	27	53
Non-Infected	-	-	-	-	-	-	NA	-	-	-	23	26	49
Non-Infected	-	-	-	-	NA	-	-	-	NA	NA	17	28	45
Non-Infected	-	-	-	-	-	-	NA	-	-	NA	3	37	40
Non-Infected	-	-	-	-	-	-	-	-	NA	-	5	32	37
Non-Infected	-	-	-	-	-	-	-	-	NA	NA	10	18	28
Non-Infected	-	-	-	-	-	NA	NA	-	-	-	1	18	19
Non-Infected	NA	NA	-	-	-	NA	-	-	-	-	0	16	16
Non-Infected	-	-	-	-	-	NA	-	NA	-	-	2	13	15
Non-Infected	-	-	+	-	-	-	-	-	-	-	8	6	14
Non-Infected	-	-	-	-	-	NA	-	-	-	NA	1	12	13
Non-Infected	-	-	-	-	-	-	-	+	-	-	7	5	12
Non-Infected	-	-	-	-	-	-	+	-	-	-	4	7	11
Non-Infected	-	-	-	-	-	NA	NA	-	-	NA	1	9	10
Non-Infected	NA	-	-	-	-	-	-	-	-	-	3	7	10
Non-Infected	-	-	-	-	-	+	-	-	-	-	7	2	9
Non-Infected	+	-	-	-	-	-	-	-	-	-	2	5	7
Non-Infected	-	-	-	+	-	-	-	-	-	-	4	3	7
Non-Infected	-	-	-	-	-	-	-	-	-	+	1	6	7
Non-Infected	-	-	-	-	+	-	-	-	-	-	1	5	6
Non-Infected	-	-	-	-	-	NA	NA	NA	-	-	3	3	6
Non-Infected	-	-	-	-	-	-	-	-	+	-	2	3	5
Non-Infected	-	+	-	-	-	-	-	-	-	-	0	4	4
Non-Infected	-	-	-	-	-	-	NA	-	NA	NA	1	2	3
Non-Infected	-	-	-	NA	-	-	-	-	-	-	2	1	3
Non-Infected	-	-	-	-	+	-	-	-	+	-	0	2	2
Non-Infected	-	-	NA	-	-	-	-	-	-	-	0	2	2
Non-Infected	-	NA	-	-	-	-	-	-	-	-	1	1	2
Non-Infected	NA	NA	-	-	-	NA	-	-	NA	-	0	2	2
Non-Infected	+	+	-	-	-	+	-	+	-	+	1	0	1
Non-Infected	+	-	-	-	+	+	-	+	+	+	1	0	1
Non-Infected	+	-	-	-	+	-	-	+	+	+	1	0	1
Non-Infected	+	-	-	-	-	+	-	+	-	-	0	1	1
Non-Infected	+	-	-	-	-	+	-	-	-	-	1	0	1
Non-Infected	-	+	-	-	-	-	NA	-	-	-	1	0	1
Non-Infected	-	-	+	+	+	+	+	+	+	+	0	1	1

Patient Infected Status	NAAT1a		NAAT2a			cobas CT/NG v2.0 Test					Symptom Status		Total
	SW	UR	SW	UR	PC Pre	SW	UR	VG	PC Pre	PC Post	Symp	Asymp	
Non-Infected	-	-	+	+	-	+	+	+	-	-	1	0	1
Non-Infected	-	-	+	+	-	-	-	+	-	-	1	0	1
Non-Infected	-	-	+	+	-	-	-	-	+	-	0	1	1
Non-Infected	-	-	-	+	+	-	+	+	+	-	0	1	1
Non-Infected	-	-	-	+	-	-	+	-	-	-	1	0	1
Non-Infected	-	-	+	-	+	-	-	+	+	+	0	1	1
Non-Infected	-	-	+	-	-	-	-	+	-	-	1	0	1
Non-Infected	-	-	+	-	-	-	-	-	-	+	1	0	1
Non-Infected	-	-	+	-	-	-	NA	NA	-	-	1	0	1
Non-Infected	-	-	-	-	+	+	-	-	-	+	1	0	1
Non-Infected	-	-	-	-	+	-	-	-	-	NA	0	1	1
Non-Infected	-	-	-	-	+	-	-	NA	-	-	0	1	1
Non-Infected	-	-	-	-	-	+	+	-	-	-	0	1	1
Non-Infected	-	-	-	-	-	+	-	+	-	-	1	0	1
Non-Infected	-	-	-	-	-	-	-	+	+	-	0	1	1
Non-Infected	-	-	-	-	-	-	-	+	-	NA	1	0	1
Non-Infected	-	-	-	-	-	-	-	NA	-	+	0	1	1
Non-Infected	-	-	-	-	-	-	-	NA	-	NA	0	1	1
Non-Infected	-	-	-	-	-	-	NA	-	+	NA	0	1	1
Non-Infected	-	-	-	-	-	-	NA	NA	-	-	0	1	1
Non-Infected	-	-	-	-	-	NA	-	NA	NA	NA	1	0	1
Non-Infected	-	-	-	-	-	NA	NA	-	NA	-	0	1	1
Non-Infected	-	-	-	-	-	NA	NA	-	NA	NA	1	0	1
Non-Infected	-	-	-	-	-	NA	NA	NA	-	NA	1	0	1
Non-Infected	-	-	NA	-	NA	-	-	-	NA	NA	1	0	1
Non-Infected	-	-	-	NA	NA	NA	NA	NA	-	-	1	0	1
Non-Infected	-	NA	-	-	-	-	-	-	-	NA	1	0	1
Non-Infected	NA	NA	-	-	-	-	-	-	-	-	0	1	1
Total Non-Infected											**1814**	**3086**	**4900**

a NAAT1 and NAAT2 = Commercially available CT/NG Assays.
b Symp = symptomatic, Asymp = asymptomatic.
Note: Subjects are designated as being infected with CT if at least two predicate NAATs with different target regions give positive results in the endocervical swab and/or urine specimen. However, females are categorized as non-infected for any swab specimen if the swab specimen and the PreservCyt specimen (NAAT2) were negative and the urine specimen was positive.
Note: + denotes Positive; - denotes Negative; NA indicates specimen was not obtained or available for testing.
Note: SW = endocervical swab; UR = urine; VG = vaginal swab; PC Pre = PreservCyt (pre-aliquot);
PC Post = PreservCyt (post-aliquot).

CT: Positive/Negative Analysis for Male Patient Infected Status

Patient Infected Status	NAAT1[a]		NAAT2[a]		cobas CT/NG v2.0 Test	Symptom Status[b]		
	SW	UR	SW	UR	UR	Symp	Asymp	Total
Infected	+	+	+	+	+	64	43	107
Infected	-	+	-	+	+	3	3	6
Infected	-	+	+	+	+	0	3	3
Infected	+	+	+	-	+	1	1	2
Infected	+	-	+	-	-	0	1	1
Infected	+	-	+	+	+	0	1	1
Infected	-	+	+	-	+	1	0	1
Infected	-	+	-	+	-	1	0	1
Total Infected						**70**	**52**	**122**
Non-Infected	-	-	-	-	-	203	399	602
Non-Infected	-	-	-	-	+	1	2	3
Non-Infected	-	-	+	-	-	1	1	2
Non-Infected	-	-	-	+	-	1	1	2
Non-Infected	-	-	+	+	-	0	2	2
Non-Infected	-	+	-	-	-	0	2	2
Non-Infected	-	-	+	+	+	0	1	1
Non-Infected	+	-	-	-	-	1	0	1
Non-Infected	+	+	-	-	+	1	0	1
Total Non-Infected						**208**	**408**	**616**

[a] NAAT1 and NAAT2 = Commercially available CT/NG Assays

[b] Symp = symptomatic, Asymp = asymptomatic.

Note: Subjects are designated as being infected with CT if at least two predicate NAATs with different target regions give positive results in the urethral swab and/or the urine specimen.

Note: + denotes Positive; - denotes Negative.

Note: SW = urethral swab, UR= urine.

Sensitivity, specificity, and predictive values of the **cobas** CT/NG v2.0 Test for CT as defined by PIS are presented by gender, sample type, and symptom status in the following table. Overall Sensitivity ranged from 93.7% to 98.4% and overall specificity ranged from 98.8% to 99.8% in both females and males. Performance estimates for CT detection were similar between symptomatic and asymptomatic subjects.

CT: Clinical Performance Compared With Patient Infected Status by Gender and Sample Type, and Symptom Status

Sample Type[a]	Symptom Status[b]	Total (n)	SENS	95% CI	SPEC	95% CI	PREV (%)	PPV (%)	NPV (%)
Female									
SW	Symp[c]	1932	94.7% (144/152)	(90.0%, 97.3%)	99.3% (1767/1780)	(98.8%, 99.6%)	7.9	91.7	99.5
	Asymp[d]	994	95.3% (81/85)	(88.5%, 98.2%)	99.6% (905/909)	(98.9%, 99.8%)	8.6	95.3	99.6
	Overall	2926	94.9% (225/237)	(91.4%, 97.1%)	99.4% (2672/2689)	(99.0%, 99.6%)	8.1	93.0	99.6
UR	Symp[c]	1937	94.4% (151/160)	(89.7%, 97.0%)	99.7% (1771/1777)	(99.3%, 99.8%)	8.3	96.2	99.5
	Asymp[d]	1008	93.3% (84/90)	(86.2%, 96.9%)	99.5% (913/918)	(98.7%, 99.8%)	8.9	94.4	99.3
	Overall	2945	94.0% (235/250)	(90.3%, 96.3%)	99.6% (2684/2695)	(99.3%, 99.8%)	8.5	95.5	99.4
VG-C	Symp[c]	899	96.2% (76/79)	(89.4%, 98.7%)	98.8% (810/820)	(97.8%, 99.3%)	8.8	88.4	99.6
	Asymp[d]	1003	100.0% (89/89)	(95.9%, 100.0%)	99.5% (909/914)	(98.7%, 99.8%)	8.9	94.7	100.0
	Overall	1902	98.2% (165/168)	(94.9%, 99.4%)	99.1% (1719/1734)	(98.6%, 99.5%)	8.8	91.7	99.8
VG-S	Symp[c]	1041	98.7% (76/77)	(93.0%, 99.8%)	99.2% (956/964)	(98.4%, 99.6%)	7.4	90.5	99.9
	Asymp[c]	996	96.0% (48/50)	(86.5%, 98.9%)	99.4% (940/946)	(98.6%, 99.7%)	5.0	88.9	99.8
	Overall	2037	97.6% (124/127)	(93.3%, 99.2%)	99.3% (1896/1910)	(98.8%, 99.6%)	6.2	89.9	99.8
PC Pre	Symp[c]	1935	94.1% (143/152)	(89.1%, 96.9%)	99.7% (1778/1783)	(99.3%, 99.9%)	7.9	96.6	99.5
	Asymp[d]	1002	94.3% (83/88)	(87.4%, 97.5%)	99.8% (912/914)	(99.2%, 99.9%)	8.8	97.6	99.5
	Overall	2937	94.2% (226/240)	(90.4%, 96.5%)	99.7% (2690/2697)	(99.5%, 99.9%)	8.2	97.0	99.5
PC Post	Symp[c]	1871	93.9% (139/148)	(88.8%, 96.8%)	99.5% (1715/1723)	(99.1%, 99.8%)	7.9	94.6	99.5
	Asymp[d]	1007	93.3% (83/89)	(86.1%, 96.9%)	99.5% (913/918)	(98.7%, 99.8%)	8.8	94.3	99.3
	Overall	2878	93.7% (222/237)	(89.8%, 96.1%)	99.5% (2628/2641)	(99.2%, 99.7%)	8.2	94.5	99.4
Male									
UR	Symp[c]	278	98.6% (69/70)	(92.3%, 99.7%)	99.0% (206/208)	(96.6%, 99.7%)	25.2	97.2	99.5
	Asymp[c]	460	98.1% (51/52)	(89.9%, 99.7%)	99.3% (405/408)	(97.9%, 99.7%)	11.3	94.4	99.8

Sample Type[a]	Symptom Status[b]	Total (n)	SENS	95% CI	SPEC	95% CI	PREV (%)	PPV (%)	NPV (%)
	Overall	738	98.4% (120/122)	(94.2%, 99.5%)	99.2% (611/616)	(98.1%, 99.7%)	16.5	96.0	99.7

[a] SW = endocervical swab, UR = urine, VG-C = clinician-collected vaginal swab, VG-S = self-collected vaginal swab, PC Pre = PreservCyt (pre-aliquot), PC Post = PreservCyt (post-aliquot).
[b] Symp = symptomatic, Asymp = asymptomatic.
[c] **cobas** CT/NG v2.0 Test results from archived specimens.
[d] **cobas** CT/NG v2.0 Test results from prospectively collected specimens.
Note: Subjects are designated as being infected with CT if at least 2 NAATs with different target regions give positive results in the endocervical swab (urethral swab for males) and/or the urine specimen. However, females are categorized as non-infected for any swab specimen if the swab specimens and the PreservCyt specimen (NAAT2) were negative and the urine specimens were positive.
Note: CI = (score) confidence interval, PREV = prevalence, SENS = sensitivity, SPEC = specificity, PPV = positive predictive value, NPV = negative predictive value.

For archived specimens from female asymptomatic patients, sensitivity for CT was 91.2% (93/102), 92.9% (104/112), 94.4% (51/54), 88.6% (93/105), and 87.5% (91/104), and specificity for CT was 99.9% (2,076/2,078), 99.8% (2,065/2,070), 99.9% (1,183/1,184), 99.6% (2,085/2,094), and 99.7% (1,881/1,886) respectively for endocervical swabs, female urine specimens, clinician-collected vaginal swabs, and PreservCyt specimens (pre- and post-ThinPrep processing).

Clinical Study Results for *Neisseria gonorrhoeae* (NG)

The following two tables summarize the results from symptomatic and asymptomatic female and male subjects designated as infected or non-infected with NG according to the PIS algorithm. A total of 92 females and 67 males were infected with NG. Symptoms were reported in 46.7% (43/92) of infected and 37.4% (1,932/5,171) of non-infected women. Similarly, symptoms were reported in 89.6% (60/67) of infected and 32.5% (218/671) of non-infected men. Overall, the NG prevalence was 1.7% (92/5,263), 9.1% (67/738), and 2.6% (159/6,001), respectively, in women, men, and the entire study population.

NG: Positive/Negative Analysis for Female Patient Infected Status

Patient Infected Status	NAAT1[a]		NAAT2[a]			cobas CT/NG v2.0 Test					Symptom Status[b]		Total
	SW	UR	SW	UR	PC Pre	SW	UR	VG	PC Pre	PC Post	Symp	Asymp	
Infected	+	+	+	+	+	+	+	+	+	+	29	37	66
Infected	+	+	+	+	+	+	+	NA	+	+	1	2	3
Infected	+	+	+	-	+	+	+	+	+	+	1	2	3
Infected	+	-	+	-	+	+	-	+	+	+	3	0	3
Infected	+	+	+	+	+	NA	+	+	+	+	1	1	2
Infected	+	+	+	+	-	+	+	+	+	+	0	2	2

Patient Infected Status	NAAT1[a]		NAAT2[a]			cobas CT/NG v2.0 Test					Symptom Status[b]		
	SW	UR	SW	UR	PC Pre	SW	UR	VG	PC Pre	PC Post	Symp	Asymp	Total
Infected	+	+	+	+	+	+	+	+	+	-	1	0	1
Infected	+	+	+	+	+	+	+	+	-	-	0	1	1
Infected	+	+	+	+	+	+	+	-	+	+	1	0	1
Infected	+	+	+	+	+	+	-	+	+	+	1	0	1
Infected	+	+	+	+	+	+	NA	+	+	+	1	0	1
Infected	+	+	+	+	+	NA	NA	NA	+	+	0	1	1
Infected	+	+	+	+	-	-	+	+	+	-	1	0	1
Infected	+	-	+	-	+	+	+	+	+	+	1	0	1
Infected	+	-	+	-	-	+	+	+	+	+	1	0	1
Infected	-	+	+	+	+	+	+	+	+	+	0	1	1
Infected	-	+	+	+	+	+	+	+	-	NA	0	1	1
Infected	-	+	+	+	+	-	+	+	+	+	1	0	1
Infected	-	+	+	+	+	-	+	+	-	-	0	1	1
Total Infected											**43**	**49**	**92**
Non-Infected	-	-	-	-	-	-	-	-	-	-	1704	2709	4413
Non-Infected	-	-	-	-	-	-	-	-	-	NA	62	177	239
Non-Infected	-	-	-	-	-	NA	-	-	-	-	30	36	66
Non-Infected	-	-	-	-	-	-	-	NA	-	-	26	29	55
Non-Infected	-	-	-	-	-	-	NA	-	-	-	24	25	49
Non-Infected	-	-	-	-	NA	-	-	-	NA	NA	18	30	48
Non-Infected	-	-	-	-	-	-	-	-	NA	-	7	37	44
Non-Infected	-	-	-	-	-	-	NA	-	-	NA	3	38	41
Non-Infected	-	-	-	-	-	-	-	-	NA	NA	10	19	29
Non-Infected	-	-	-	-	-	NA	NA	-	-	-	1	18	19
Non-Infected	-	-	-	-	-	NA	-	NA	-	-	2	15	17
Non-Infected	NA	NA	-	-	-	NA	-	-	-	-	0	16	16
Non-Infected	-	-	-	-	-	-	+	-	-	-	5	9	14
Non-Infected	-	-	-	-	-	NA	-	-	-	NA	1	12	13
Non-Infected	+	-	-	-	-	-	-	-	-	-	5	6	11
Non-Infected	-	-	-	-	-	NA	NA	-	-	NA	1	9	10
Non-Infected	NA	-	-	-	-	-	-	-	-	-	3	7	10
Non-Infected	-	+	-	-	-	-	-	-	-	-	0	9	9

Patient Infected Status	NAAT1[a]		NAAT2[a]			cobas CT/NG v2.0 Test					Symptom Status[b]		
	SW	UR	SW	UR	PC Pre	SW	UR	VG	PC Pre	PC Post	Symp	Asymp	Total
Non-Infected	-	-	-	-	-	-	-	-	-	+	3	4	7
Non-Infected	-	-	-	-	-	NA	NA	NA	-	-	3	2	5
Non-Infected	-	-	-	+	-	-	-	-	-	-	2	2	4
Non-Infected	-	-	-	-	-	-	-	+	-	-	2	2	4
Non-Infected	-	-	NA	-	-	-	-	-	-	-	2	2	4
Non-Infected	-	-	-	-	+	-	-	-	-	-	1	2	3
Non-Infected	-	-	-	-	-	-	-	-	+	-	1	2	3
Non-Infected	-	-	-	-	-	-	NA	-	NA	NA	1	2	3
Non-Infected	-	-	-	NA	-	-	-	-	-	-	2	1	3
Non-Infected	-	-	+	-	-	-	-	-	-	-	1	1	2
Non-Infected	-	-	-	-	-	-	-	+	-	+	0	2	2
Non-Infected	-	-	-	-	-	-	-	NA	-	NA	0	2	2
Non-Infected	-	-	-	-	-	-	NA	NA	-	-	1	1	2
Non-Infected	-	NA	-	-	-	-	-	-	-	-	1	1	2
Non-Infected	NA	NA	-	-	-	NA	-	-	NA	-	0	2	2
Non-Infected	+	+	-	-	+	+	+	+	+	+	0	1	1
Non-Infected	+	+	-	-	+	NA	+	+	+	+	0	1	1
Non-Infected	+	+	-	-	-	-	-	-	-	-	0	1	1
Non-Infected	+	-	-	-	-	+	NA	-	-	-	0	1	1
Non-Infected	-	+	-	-	-	-	-	-	-	NA	0	1	1
Non-Infected	-	-	-	-	+	-	-	-	+	-	0	1	1
Non-Infected	-	-	-	-	-	+	-	-	-	-	1	0	1
Non-Infected	-	-	-	-	-	+	-	-	-	NA	0	1	1
Non-Infected	-	-	-	-	-	+	-	-	NA	NA	1	0	1
Non-Infected	-	-	-	-	-	-	+	-	NA	-	0	1	1
Non-Infected	-	-	-	-	-	-	-	+	-	NA	1	0	1
Non-Infected	-	-	-	-	-	NA	-	-	+	+	1	0	1
Non-Infected	-	-	-	-	-	NA	-	NA	NA	NA	1	0	1
Non-Infected	-	-	-	-	-	NA	NA	-	NA	-	0	1	1
Non-Infected	-	-	-	-	-	NA	NA	-	NA	NA	1	0	1
Non-Infected	-	-	-	-	-	NA	NA	NA	-	NA	1	0	1
Non-Infected	-	-	-	-	NA	NA	-	-	NA	NA	1	0	1

Patient Infected Status	NAAT1[a]		NAAT2[a]			cobas CT/NG v2.0 Test					Symptom Status[b]		Total
	SW	UR	SW	UR	PC Pre	SW	UR	VG	PC Pre	PC Post	Symp	Asymp	
Non-Infected	-	-	-	NA	NA	NA	NA	NA	-	-	1	0	1
Non-Infected	-	NA	-	-	-	-	-	-	-	NA	1	0	1
Non-Infected	NA	NA	-	-	-	-	-	-	-	-	0	1	1
Total Non-Infected											**1932**	**3239**	**5171**

a NAAT1 and NAAT2 = Commercially available CT/NG Assays.
b Symp = symptomatic, Asymp = asymptomatic.
Note: Subjects are designated as being infected with NG if at least two predicate NAATs with different target regions give positive results in the endocervical swab and/or urine specimen.
Note: + denotes Positive; - denotes Negative; NA indicates specimen was not obtained or available for testing.
Note: SW = endocervical swab; UR = urine; VG = vaginal swab; PC Pre = PreservCyt (pre-aliquot); PC Post = PreservCyt (post-aliquot).

NG: Positive/Negative Analysis for Male Patient Infected Status

Patient Infected Status	NAAT1[a]		NAAT2[a]		cobas CT/NG v2.0 Test	Symptom Status[b]		Total
	SW	UR	SW	UR	UR	Symp	Asymp	
Infected	+	+	+	+	+	59	7	66
Infected	+	+	-	+	+	1	0	1
Total Infected						**60**	**7**	**67**
Non-Infected	-	-	-	-	-	213	449	662
Non-Infected	-	-	-	-	+	2	3	5
Non-Infected	-	+	-	-	-	1	1	2
Non-Infected	-	-	+	-	-	1	0	1
Non-Infected	+	-	-	-	-	1	0	1
Total Non-Infected						**218**	**453**	**671**

[a] NAAT1 and NAAT2 = Commercially available CT/NG Assays
[b] Symp = symptomatic, Asymp = asymptomatic.
Note: Subjects are designated as being infected with NG if at least two predicate NAATs with different target regions give positive results if at least in the urethral swab and/or the urine specimen.
Note: + denotes Positive; - denotes Negative.
Note: SW = urethral swab, UR= urine.[a]

Sensitivity, specificity, and predictive values of the **cobas** CT/NG v2.0 Test for NG as defined by PIS are shown by gender, sample type, and symptom status in the following table. Overall sensitivity ranged from 95.6% to 100.0%. Overall specificity ranged from 99.1% to 100.0% for both females and males. Performance estimates for NG detection

were similar between symptomatic and asymptomatic subjects.

NG: Clinical Performance Compared With Patient Infected Status by Gender, Sample Type, and Symptom Status

Sample Type[a]	Symptom Status[b]	Total (n)	SENS	95% CI	SPEC	95% CI	PREV (%)	PPV (%)	NPV (%)
Female									
SW	Symp[c]	1930	95.2% (40/42)	(84.2%, 98.7%)	99.9% (1886/1888)	(99.6%, 100.0%)	2.2	95.2	99.9
	Asymp[d]	3174	97.9% (46/47)	(88.9%, 99.6%)	99.9% (3124/3127)	(99.7%, 100.0%)	1.5	93.9	100.0
	Overall	5104	96.6% (86/89)	(90.6%, 98.8%)	99.9% (5010/5015)	(99.8%, 100.0%)	1.7	94.5	99.9
UR	Symp[c]	1937	90.5% (38/42)	(77.9%, 96.2%)	99.7% (1890/1895)	(99.4%, 99.9%)	2.2	88.4	99.8
	Asymp[d]	3190	100.0% (48/48)	(92.6%, 100.0%)	99.6% (3130/3142)	(99.3%, 99.8%)	1.5	80.0	100.0
	Overall	5127	95.6% (86/90)	(89.1%, 98.3%)	99.7% (5020/5037)	(99.5%, 99.8%)	1.8	83.5	99.9
VG-C	Symp[c]	898	100.0% (21/21)	(84.5%, 100.0%)	99.7% (874/877)	(99.0%, 99.9%)	2.3	87.5	100.0
	Asymp[d]	2240	100.0% (37/37)	(90.6%, 100.0%)	99.7% (2197/2203)	(99.4%, 99.9%)	1.7	86.0	100.0
	Overall	3138	100.0% (58/58)	(93.8%, 100.0%)	99.7% (3071/3080)	(99.4%, 99.8%)	1.8	86.6	100.0
VG-S	Symp[c]	1041	95.2% (20/21)	(77.3%, 99.2%)	100.0% (1020/1020)	(99.6%, 100.0%)	2.0	100.0	99.9
	Asymp[c]	996	100.0% (9/9)	(70.1%, 100.0%)	100.0% (987/987)	(99.6%, 100.0%)	0.9	100.0	100.0
	Overall	2037	96.7% (29/30)	(83.3%, 99.4%)	100.0% (2007/2007)	(99.8%, 100.0%)	1.5	100.0	100.0
PC Pre	Symp[c]	1935	100.0% (43/43)	(91.8%, 100.0%)	99.9% (1890/1892)	(99.6%, 100.0%)	2.2	95.6	100.0
	Asymp[d]	3196	93.9% (46/49)	(83.5%, 97.9%)	99.8% (3142/3147)	(99.6%, 99.9%)	1.5	90.2	99.9
	Overall	5131	96.7% (89/92)	(90.8%, 98.9%)	99.9% (5032/5039)	(99.7%, 99.9%)	1.8	92.7	99.9
PC Post	Symp[c]	1872	95.3% (41/43)	(84.5%, 98.7%)	99.8% (1825/1829)	(99.4%, 99.9%)	2.3	91.1	99.9
	Asymp[d]	2996	95.8% (46/48)	(86.0%, 98.8%)	99.7% (2940/2948)	(99.5%, 99.9%)	1.6	85.2	99.9
	Overall	4868	95.6% (87/91)	(89.2%, 98.3%)	99.7% (4765/4777)	(99.6%, 99.9%)	1.9	87.9	99.9
Male									
UR	Symp[c]	278	100.0% (60/60)	(94.0%, 100.0%)	99.1% (216/218)	(96.7%, 99.7%)	21.6	96.8	100.0

Sample Type[a]	Symptom Status[b]	Total (n)	SENS	95% CI	SPEC	95% CI	PREV (%)	PPV (%)	NPV (%)
	Asymp[c]	460	100.0% (7/7)	(64.6%, 100.0%)	99.3% (450/453)	(98.1%, 99.8%)	1.5	70.0	100.0
	Overall	738	100.0% (67/67)	(94.6%, 100.0%)	99.3% (666/671)	(98.3%, 99.7%)	9.1	93.1	100.0

[a] SW = endocervical swab, UR = urine, VG-C = clinician-collected vaginal swab, VG-S = self-collected vaginal swab, PC Pre = PreservCyt (pre-aliquot), PC Post = PreservCyt (post-aliquot).
[b] Symp = symptomatic, Asymp = asymptomatic.
[c] **cobas** CT/NG v2.0 Test results from archived specimens.
[d] **cobas** CT/NG v2.0 Test results from prospectively collected specimens.
Note: Subjects are designated as being infected with CT if at least 2 NAATs with different target regions give positive results in the endocervical swab (urethral swab for males) and/or the urine specimen.
Note: Subjects with designated infection status and valid **cobas** CT/NG v2.0 Test results are considered evaluable and included in this summary table.
Note: CI = (score) confidence interval, PREV = prevalence, SENS = sensitivity, SPEC = specificity, PPV = positive predictive value, NPV = negative predictive value.

4. Clinical cut-off**:**

   Not applicable

5. Expected values/Reference range:

   For the various collection sites in the multi-center clinical study, the prevalence of CT observed with the **cobas** CG/NG v2.0 Test ranged from 5.0% to 8.9% in females, and from 11.3% to 25.2% in males. The prevalence for NG ranged from 0.9% to 2.3% in females and from 1.5% to 21.6% in males.

   The following two tables illustrate hypothetical positive and negative predictive values for the detection of CT and NG with the **cobas** CT/NG v2.0 Test for potential prevalence of 1 to 50 %. The overall sensitivity and specificity used for these calculations were derived from the clinical study results for all specimen types combined.

Positive and Negative Predictive Values for Hypothetical CT Prevalence

Prevalence (%)	Sensitivity* (%)	Specificity* (%)	PPV (%)	NPV (%)
1	94.1	99.6	69.0	99.9
3	94.1	99.6	87.2	99.8
5	94.1	99.6	92.0	99.7
10	94.1	99.6	96.1	99.3
15	94.1	99.6	97.5	99.0
20	94.1	99.6	98.2	98.5
30	94.1	99.6	98.9	97.5
50	94.1	99.6	99.5	94.4

Prevalence (%)	Sensitivity* (%)	Specificity* (%)	PPV (%)	NPV (%)

* Overall sensitivity and specificity were estimated by comparing the **cobas**® CT/NG v2.0 Test results to patient infected status across all sample types in both female and male subjects.
Note: PPV = positive predictive value; NPV = negative predictive value.

Positive and Negative Predictive Values for Hypothetical NG Prevalence

Prevalence (%)	Sensitivity* (%)	Specificity* (%)	PPV (%)	NPV (%)
1	97.1	99.8	82.0	100.0
3	97.1	99.8	93.3	99.9
5	97.1	99.8	96.0	99.8
10	97.1	99.8	98.0	99.7
15	97.1	99.8	98.8	99.5
20	97.1	99.8	99.1	99.3
30	97.1	99.8	99.5	98.8
50	97.1	99.8	99.8	97.2

* Overall sensitivity and specificity were estimated by comparing the **cobas**® CT/NG v2.0 Test results to patient infected status across all sample types in both female and male subjects.
Note: PPV = positive predictive value; NPV = negative predictive value.

N. Instrument Name:

Roche **cobas**® 4800 System consisting of **cobas** 480 x and **cobas** 480 z instruments.

O. System Descriptions:

1. Modes of Operation:

   The Roche **cobas** 4800 System operates in a batch mode with open sample tubes. The system may operate in full extraction and amplification mode or in PCR only mode with previously extracted samples. Urine, vaginal, and endocervical specimens collected in the appropriate **cobas** collection devices may be combined in the same run. PreservCyt specimens must be run separately from the other specimen types. Each run can 24 or 96 specimens.

2. Software:

   FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

   Yes __X____ or No _____

3. Specimen Identification:

   Specimens are identified using barcodes on specimen vials.

4. Specimen Sampling and Handling:

Specimens are placed on the **cobas** x 480 instrument as open tubes and specimen processing is fully automated. After completion of specimen processing, the user transfers the plate carrier to the **cobas** z 480 instrument for automated amplification and detection. Specimens can be processed directly from primary collection vials or as aliquots of the specimen in secondary vials.

5. Calibration:

   No calibration is required by the user. Roche technicians perform calibration periodically as required.

6. Quality Control: See Section "c" above.

P. Other Supportive Instrument Performance Characteristics Data Not Covered in the "Performance Characteristics" Section above:

n/a

Q. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

R. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.